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A TEXT-BOOK

of

CLINICAL DIAGNOSIS

BY LABORATORY METHODS

FOR THE USE OF

STUDENTS, PRACTITIONERS, AND LABORATORY WORKERS

BY

L. NAPOLEON BOSTON, A.M., M.D.

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OF THE

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IN APPRECIATION OF THEIR THOROUGH WORK AND
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32088

PREFACE TO THE SECOND EDITION.

It is very gratifying to the author to be called upon to prepare a revision of his book eight months after its publication; and the favorable reception thus indicated has added stimulus to his effort to keep this volume abreast of modern advance.

In this edition slight changes and additions have been made throughout the text and seventeen pages of new matter, covering the more important advances, have been added at the end. The additions include, among other things, such important subjects as Biff's New Hemogelometer; Ficker's Reaction; a description (illustrated) of the Leishman-Donovan Bodies; Ravold's Test for Albumin; Cammidge's Test for Glycerin; Cipollino's Test, etc. The subject of Cytodiagnosis is given more extended consideration, as practical use of this method has clearly demonstrated its value. References to these addenda are made at appropriate places in the body of the book.

JULY, 1905.

PREFACE.

This book has emanated from a desire to satisfy the demands made by my pupils, and it has been prepared to meet the present needs of the student and of the general practitioner.

It is hoped that the volume may prove of practical value, although it does not attempt to furnish more than a working introduction to the department of medicine under discussion. It is, however, designed as a practical guide, and the methods described are such as can be carried out with a minimum of complicated apparatus.

Special attention has been given to outlining in progressive steps the various procedures in clinical technic, such steps being illustrated whenever possible. Besides the detailed descriptions of methods of examination, the diagnostic significance of the clinical findings is given under special headings and is frequently accompanied by tables of differentiation.

In the chapter on the blood the more recent methods for examination and staining are described and illustrated by original drawings; and the subject of Serum-diagnosis is carefully considered. The protozoan parasites known to invade the blood of the lower animals are described in connection with the malarial parasite.

In the chapter on the urine the newer methods for the estimation of sugar, Bence-Jones' albumin, uric acid, and purin have received consideration, and the whole chapter is illustrated by a large number of original half-tones and colored plates.

An unusual amount of space is given to the consideration of Animal Parasites, Diseases of the Skin, Transudates and Exudates, and the Secretions of the Eye and Ear. Inoscopy and Cytodiagnosis have been briefly considered.

I am indebted to Dr. Chas. Wardell Stiles for valuable suggestions regarding the subject of animal parasites; to Dr. John A. McKenna, who read the proof, and to W. B. Saunders & Co. for special courtesies rendered while the book was in press. In material taken from the literature I have endeavored to give credit in each instance.

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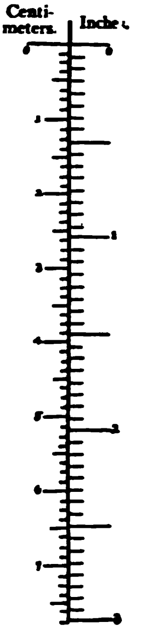
Centigrade.	Fahrenheit.	Centigrade.	Fahrenheit.
110°	230°	37°	98.6°
100	212	36.5	97.7
95	203	36	96.8
90	194	35.5	95.9
85	185	35	95.0
80	176	34	9.32
75	167	33	91.4
70	158	32	89.6
65	149	31	87.8
60	140	30	86
55	131	25	77
50	122	20	68
45	113	15	59
44	111.2	10	50
43	109.4	+5	41
42	107.6	0	32
41	105.8	—5	23
40.5	104.9	—10	14
40	104.0	—15	+5
39.5	103.1	—20	—4
39	102.2		
38.5	101.3	0.54°	= 1°
38	100.4	1	= 1.8
37.5	99.5	2	= 3.6
		2.5	= 4.5



Comparative scale in centimeters and inches.

WEIGHTS AND MEASURES.

1. English Weights and Measures.

1 grain (gr.).
1 ounce (oz.)...... = 437.5 grains.
1 pound (lb.)..... = 16 ounces = 7000 **grains.**
1 minim .... = 0.91146 grain.
1 fluidram......... = 60 minims.
1 fluidounce...... = 8 fluidrams.
1 pint .... = 20 fluidounces.
1 gallon.......... = 8 pints.

2. Relations of English to Metric Systems.

1 grain .... = 64.8 milligrams.
1 ounce.......... = 28.3 grams.
1 pound.......... = 453.6 grams.
1 gram .... = 15.432 grains.
1 kilo............ = 2 pounds 3 ounces.
1 minim .... = 0.059 cubic centimeter.
1 fluidram........ = 3.5 cubic centimeters.
1 fluidounce ...... = 28.39 cubic centimeters.
1 pint .... = 567.9 cubic centimeters.
1 c.c. .... = 16.9 minims.
1 liter .... = 35.2 fluidounces.
1 inch .... = 2.54 centimeters.
1 foot............ = 30.48 centimeters.
1 yard .... = 91.44 centimeters.
1 centimeter ...... = 0.39 inch.
1 meter .... = 39.37 inches.

CLINICAL DIAGNOSIS.

INTRODUCTION.

THE MICROSCOPE AND ITS USE.

BEFORE attempting to use the microscope the student should place the instrument upon a table, in position, and examine it carefully, comparing it with the illustration (Fig. 1), and whenever necessary, he should examine the accompanying key in order that he be thoroughly acquainted with this complicated instrument. I am firmly convinced that the chief reason the majority of men fail to obtain satisfactory results with the microscope is that they have neglected to acquire a practical knowledge of the instrument, and to familiarize themselves with the technic necessary for its clinical adoption.

In clinical microscopy the so-called compound microscope is required; and here it may be well to emphasize the fact that it is impossible to do all the forms of clinical work unless the microscope is provided with a two-thirds, a one-sixth, and a one-twelfth oil-immersion objective (Fig. 2) and a substage condenser (Fig. 2). In order to use a one-twelfth objective properly a condenser is absolutely essential—an Abbé condenser is the form usually supplied (Fig. 2, E).

THE DIAPHRAGM.

In a modern microscope the diaphragm (Fig. 2, G) is so arranged as to be readily adjustable. The so-called iris diaphragm is now employed for clinical work and has become an essential feature of the microscope. The particular function of the diaphragm is to cut off all adventitious light, thereby securing illumination of the object in such a manner that the light upon reaching the microscope is composed of those rays that come from the immediate vicinity of the object only. A diaphragm is further necessary in each substage in order that the aperture may be gaged to suit the different objectives in use, as well as the different objects to be observed. Should light be received directly from a mirror, it is well to have the diaphragm situated as close as possible to the object, in order to prevent all adventitious light from reaching the object. Again, the diaphragm will be less likely to interfere with illumination from a mirror when it is near the object illuminated. In clinical microscopy the condenser (illuminator, Fig. 2, E) is always to be employed, and for this reason a further discussion of the diaphragm appears valueless.

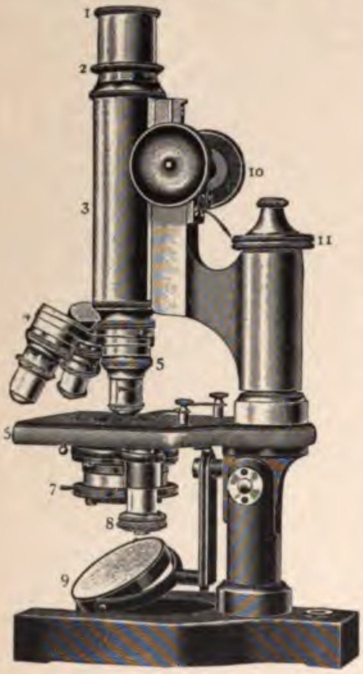


Fig. 1.—1, Huygenian ocular; 2, draw-tube, to lengthen or shorten; 3, main tube; 4, nose-piece with objectives attached; 5, objective in position; 6, stage from which substage is suspended; 7, substage; 8, adjustment of substage; 9, mirror with plain and concave faces; 10, milled head of coarse adjustment; 11, screw of fine adjustment.

LIGHT.

It is not practicable to employ unmodified light in clinical microscopy, and though it is preferable to obtain light from a northern exposure, this, too, is often impracticable. A northern exposure, when the sky is covered with white clouds, affords a most desirable light, but in this climate the microscopist must complete his work independent of climatic conditions. I have found through experience that a large portion of the microscopic study must be through the use of artificial light. A glance at figure 3 will enable the reader to obtain satisfactory light by carefully manipulating the microscope's mirror. When there are valid reasons for the operator not sitting so as to face the light, it is well to obtain light from over the left shoulder, as is done in reading. Light employed for the microscope is usually spoken of as reflected and transmitted, axial and oblique; these terms designating whether the light which illuminates a given object traverses such object or is reflected upon it; and whether or not this object is lighted uniformly or asymmetrically.

Oblique Light.—This is light in which parallel rays from a plane mirror form an angle with the optic axis of the microscope. Or if a concave mirror or a condenser is used, the light is oblique when the axial rays of the cone of light form an angle with the optic axis (Gage). (See Fig. 3.)

The Getting of Light with the Mirror.—1. Illumination is readily obtained by moving to the left the substage and condenser (Fig. 2), and by placing a mounted specimen upon the microscope stage, and employing a low objective (two-thirds) and a low ocular (Fig. 4).

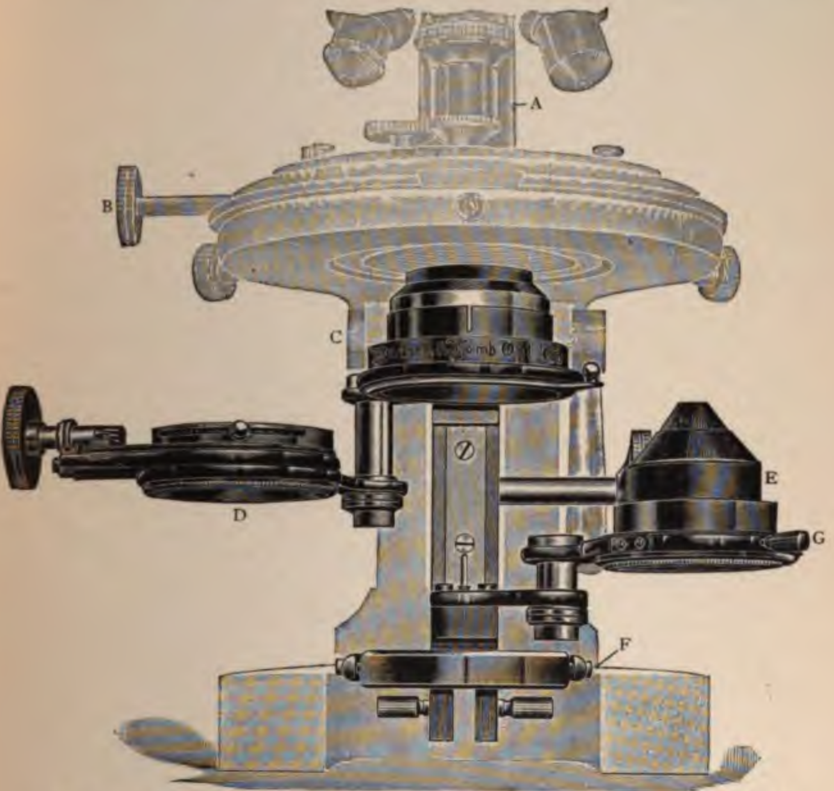


Fig. 2.—Portion of microscope showing substage separated into three parts (C, D, E): A, Objective; B, adjustment of mechanical stage; F, mirror; G, pivot to adjust iris diaphragm.

2. Do not attempt to illuminate the specimen until the objective is lowered to a point about one centimeter distant from the object.

3. Set the diaphragm so that its central opening is approximately the size of the tip of the objective.

4. Manipulate the mirror (plane surface up) until the light is reflected through the diaphragm to the objective.

5. Whenever the column of light has passed through the diaphragm, an illuminated spot appears immediately beneath the objective; and by looking into the microscope (Fig. 5), the illuminated object is seen. It is unnecessary to explain how the mirror should be manipulated in order to effect this end, since considerable practical experience is needed.

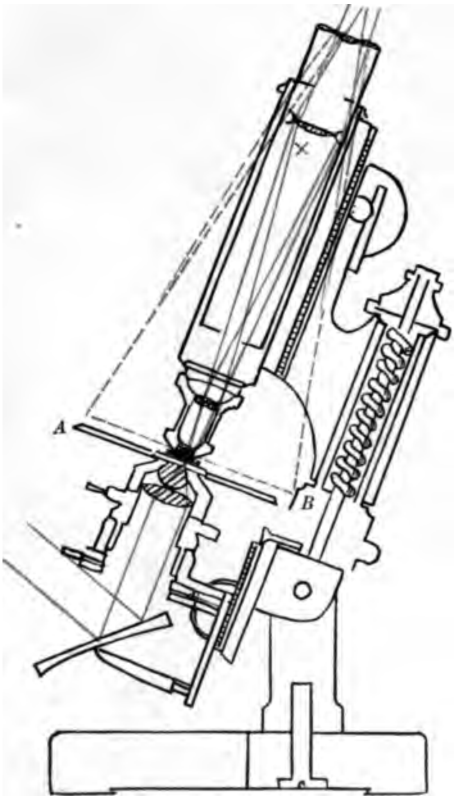


Fig. 3.—Diagram of the section of the microscope when in use, showing the rays from the mirror through the condenser to the object, and thence through the lenses of the objective to the real image and through the ocular into the eye. Dotted lines show projected rays from retinal image as the virtual image (*A*, *B*).

6. A more brilliant illumination is to be obtained through the use of the concave surface of the mirror, but in using this surface the mirror's position must be at such distance from the object that its focus will be at a level with the object.

7. It is my custom to advise all beginners in microscopy to obtain light through a low-power objective with a mounted specimen upon the stage of the microscope. The object which serves best for this purpose is a permanently mounted specimen of crystals of uric acid. These crystals are sufficiently large to be readily seen through a two-thirds objective (Plate 15), and possess sufficient color to render them conspicuous.

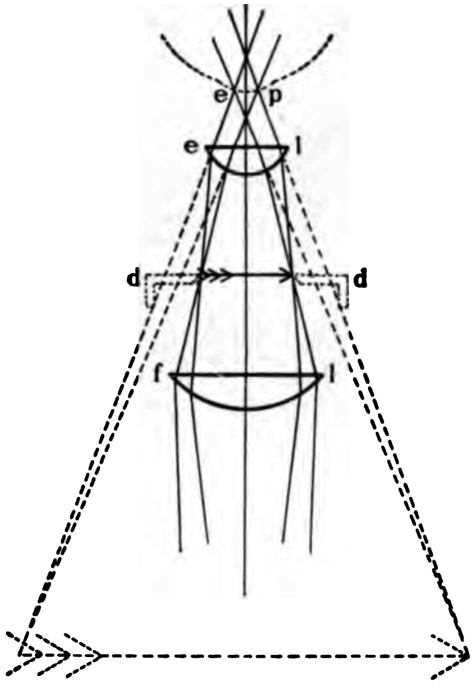


Fig. 4.—Negative eye-piece: d, d, Focal point and diaphragm; e, l, eye-lens; f, l, field-lens; e, p, rays entering cornea.

8. The position of the substage, which also governs the position of the diaphragm, is of vital importance in obtaining satisfactory illumination, and a knowledge of this important point is to be obtained only through careful manipulation of the thumbscrew of the substage (Fig. 6), while at the same time the operator closely observes the effect of such manipulation upon the field and object under study.

FOCUS.

By focusing is meant a bringing of the microscope and the object to be studied in such apposition that a clear image of the object is seen. It will be found that the higher the power of magnification, the nearer must the object be brought to the objective. It is practical, therefore, to note that with the compound clinical microscope the higher the objective, and the longer the microscope's tube, the nearer the objective must be to the object. Again, unless the oculars be par-focal, the higher the magnification of the ocular, the nearer must the object be brought to the objective. These general principles will serve to guide the beginner, though in a short time actual experience teaches him the distance above the cover-glass, or above the object, at which each objective focuses.

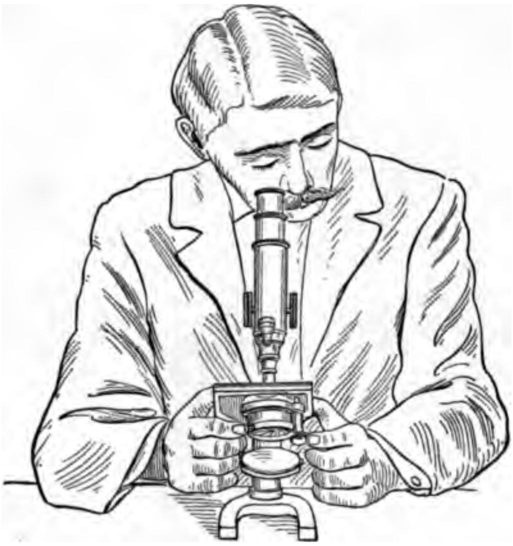


Fig. 5.—Manipulation of substage, at same time adjusting iris diaphragm.

Working Distance.—The distance from the object at which the objective of a compound microscope focuses is referred to as its working distance. This working distance will be found to vary within rather wide limits, and such variation will depend upon the power of magnification obtained; in other words, the higher the power of magnification, the shorter the working distance.

In view of these optical conditions, the thickness of the cover-glass is of importance, since it may exceed the length of the working distance of the objective employed, in which case it is impossible to focus the object. I have found that the American one-sixth objective has too short a working distance to focus upon the lines of a Thoma-Zeiss counting chamber unless a thin cover-glass be employed.

In focusing a one-sixth or an oil-immersion objective the technic differs but slightly:

1. Place the slide containing the object to be studied upon the microscope stage, and anchor it with one of the stage clamps.

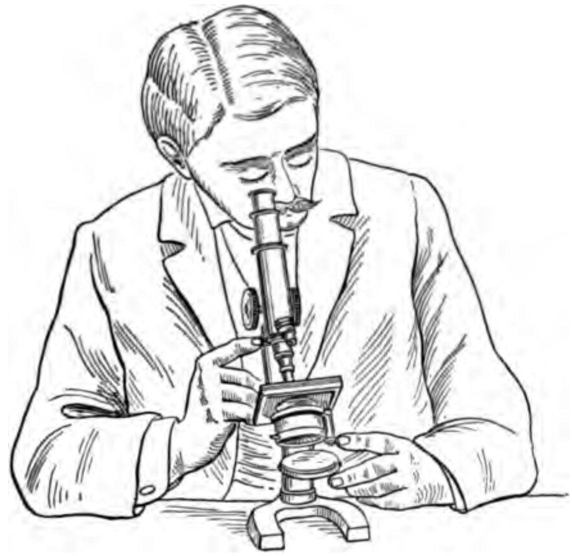


Fig. 6.—Manipulation of fine adjustment with right hand, and substage with left hand. Tube extended to 160 mm.

2. Place the head in a position so that the eye is on a level with the stage of the microscope, and with the right hand lower the tube by means of the coarse adjustment until it is possible to get but a faint ray of light between the objective and the superior surface of the cover-glass (Fig. 7).

3. The eye is now placed in position at the ocular and the tube of the microscope gently elevated by means of the coarse adjustment. In most instances it requires but a limited amount of practice to enable the student to focus readily after this method.

4. When the object springs into view, the tube is first lowered

slightly, and again elevated but a trifle, this manipulation being continued until a fairly clear outline of the object is obtained.

5. The fine adjustment wheel (see Fig. 8) should be rotated slowly, and at the same time the eye be kept in position so as to observe the slightest change in the definition.

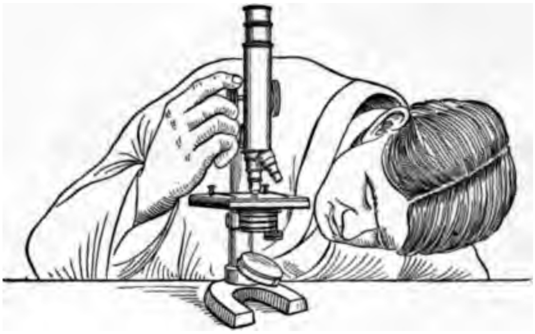


Fig. 7.—Position of operator in focusing.

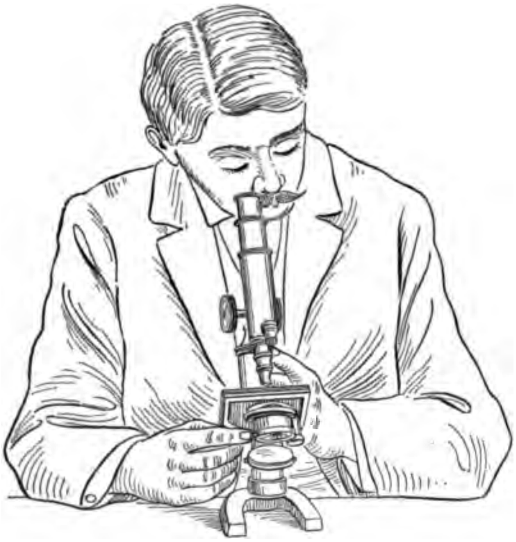


Fig. 8.—Manipulating fine adjustment with left hand and iris diaphragm with right hand.

Caution.—Do not rotate the fine adjustment wheel more than once. It will be found that this wheel, when rotated but a section of the circle, will change materially the definition (clearness of the object). Whenever the field of vision becomes dimmed to the slightest degree, the fine adjustment wheel should be rotated in the opposite direction.

The Condenser.—The condenser (Fig. 2, E) is necessary in order to do satisfactory microscopic work. When using the flat surface of the mirror, the exact position of the condenser is not of special moment; but practical experience shows that it is possible to employ the concave surface of the mirror to reflect light through the condenser; the distance between the condenser and the mirror, and the object, all become of importance. It is necessary that the operator manipulate the thumb-screw elevating and lowering the substage until the clearest definition is obtained (Fig. 6). Careful adjustment of the substage will be found of inestimable value in bringing out the lines bounding the squares of the Thoma-Zeiss counting chamber.

CARE OF THE MICROSCOPE.

In view of the fact that every microscope is provided with a small booklet giving special directions for its care, it does not appear to be necessary to do more than refer to the subject in this volume.

The location of a particle of dirt that may obstruct or interfere with the view of a certain portion of the field is of practical interest, and will therefore be considered:

1. First, while looking at an object through the microscope rotate the ocular to determine whether or not the particle that obscures the field is rotated; and should the particle rotate, it is situated in the ocular.

(*a*) Remove the ocular from the tube, and unscrew for a short distance its upper lens; return the ocular in position and rotate this lens, and should the dirt rotate with the lens, the superior lens only needs cleansing.

(*b*) The above steps are applicable to the inferior lens of the ocular.

2. Particles of lint and dust may collect upon the condenser, and the location of such dust is determined through the fact that it springs out of focus and returns into focus when the thumb-screw of the condenser is manipulated (Fig. 6).

3. Should the particle of dust remain unchanged after examining both the ocular and the condenser, the student will know that it is situated in the objective. This point may be further determined by screwing the objective to about two-thirds its depth into the nose-piece, and then rotating the objective with

the eye at the ocular, when the particle of dust will be rotated with the objective.

Cleansing the objective, the lenses of the condenser, and the lenses of the ocular is of great importance in securing clear definition. It is customary to cleanse these lenses with a special paper, and under certain conditions it is allowable to moisten such paper with some liquid.

Caution.—The lens nearest the tip of the objective is not to be cleansed with xylol, ether, or alcohol. It is my practice to cleanse all lenses of the microscope by the use of a silk or soft linen handkerchief, moistening the handkerchief with saliva. Practice has shown saliva to be the most effectual medium for the removal of oil from the objective. By moistening the finger with saliva and sweeping over the surface of a cover-glass after the use of an oil-immersion objective, the oil is instantly removed from the cover, leaving a highly polished surface. Saliva is also a valuable solvent for Canada balsam.

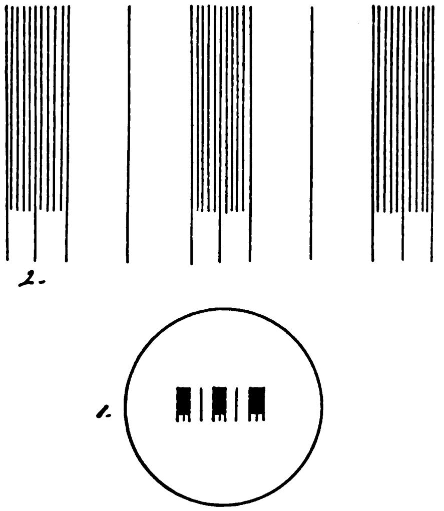


Fig. 9.—1, Ocular micrometer (natural size); 2, scale of micrometer (greatly enlarged).

MEASURING OF SPECIMENS.

The measuring of microscopic objects is known as *micrometry.* It would seem advisable to outline a method by which even the inexperienced may be able to determine the size of certain objects studied. The special apparatus necessary for this purpose is an ocular micrometer (eye-piece micrometer). This apparatus consists of a thin ground glass, upon one surface of which is placed a graduated scale composed of from fifty to one hundred lines (Fig. 9).

The ocular micrometer is placed in the eye-piece upon the diaphragm, graduated side down. In the study of objects these fine lines appear to the eye as though ground upon the microscopic field. By rotating the ocular the graduated scale is shifted to any portion of the field; thus it is possible to place the lines directly over the object to be measured (Fig. 10).

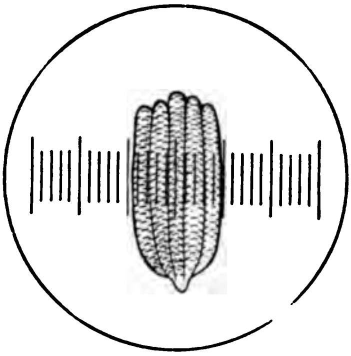


Fig. 10.—Method of using eye-piece micrometer.

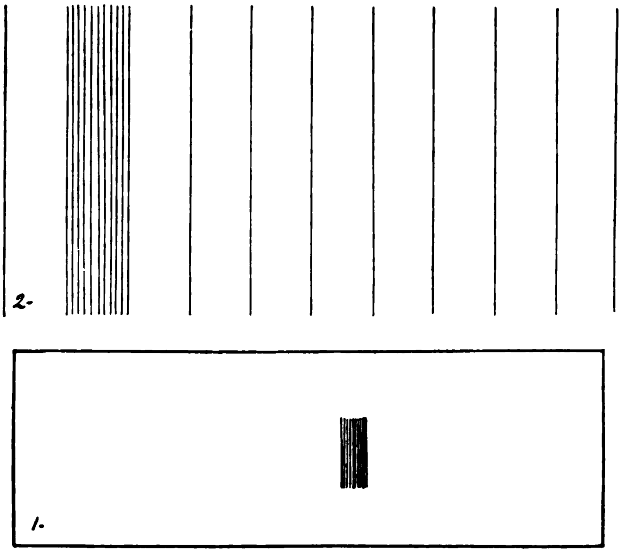


Fig. 11.—1, Stage micrometer, showing graduation at center (natural size); 2, graduation of stage micrometer (greatly enlarged).

Let us suppose that it requires twenty-five of these lines of the graduation to equal the length of the object, a one-fifth objective being employed. Remove this one-fifth objective and place in its stead a one-sixth objective. It will now be found that thirty or more of the lines of the eye-piece micrometer are necessary to equal the length of the object. In other words, the number of lines required for a certain object depends entirely upon the magnification accomplished by the objective employed. The ocular micrometer enables one to determine the length or breadth of a given microscopic object when this object is subjected to a definite degree of magnification.

The value of a definite number of lines graduated on the eye-piece micrometer that were found to equal the length of a given object is determined through the use of a stage micrometer (Fig. 11). Upon the center of this glass slide is seen a graduation in hundredths and thousandths of an inch. The stage micrometer is now to be subjected to the same degree of magnification that has been employed for the study of the object to be measured. The question of determining the measurement of the object in inches is but a matter of ascertaining what fractional portion of an inch on the stage micrometer is equivalent to the given number of lines of the ocular micrometer. This is best accomplished by rotating the ocular micrometer until its lines are parallel with the lines on the stage micrometer.

The measurement of bacteria is scarcely practicable for clinical research, and the above-outlined method is given more particularly for the measurement of animal parasites and their ova.

RINGING OF SPECIMENS.

The ringing of microscopic specimens (surrounding the cover-glass with a narrow band of microscopic cement) is employed whenever it is desired that a specimen be kept for an indefinite period. Specimens that are to be ringed should be mounted in a liberal quantity of Canada balsam, in order that the balsam extend just beyond the margin of the cover-glass at all points of its periphery. The accompanying illustration (Fig. 12) will serve to describe the instrument employed for this purpose.

Anchor the slide to be ringed upon the revolving table of the ringer by means of clamps, care being taken to shift the slide so that its center is at the center of the table (see fine lines, Fig. 12). Grasp a small camel's-hair brush between the thumb and index-finger of the left hand, and dip the tip of the brush into the microscopic cement and then rest the wrist firmly upon the ringer, allowing the tip of the brush to near the margin of the cover-glass. Hold the brush steadily, and rotate the circular table of the ringer rapidly; then lower the tip of the brush to touch the margin of the cover.

Caution.—The circular table must be rotated with rapidity, and a liberal amount of cement be present upon the tip of the brush. It has been my custom to place the ringer upon its side (the slide placed in position), and to press the small wheel of the ringer against the moving band of a sewing-machine.

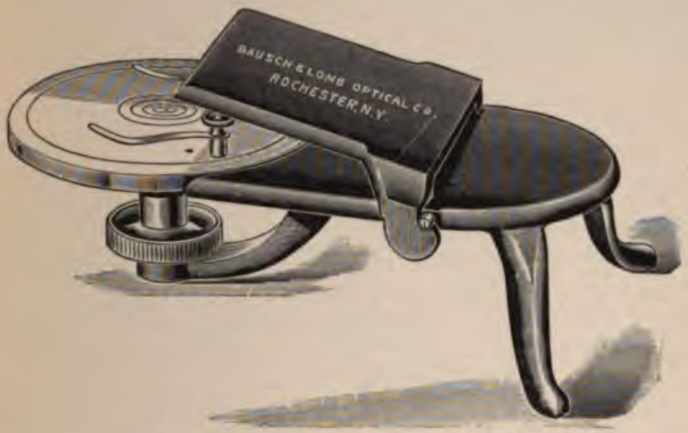

BAUSCH & LOMB OPTICAL CO.
ROCHESTER, N.Y.

Fig. 12.—Turntable for ringing mounted slides.

Specimen slides ringed in this manner have been in my collection for a period of seven years.

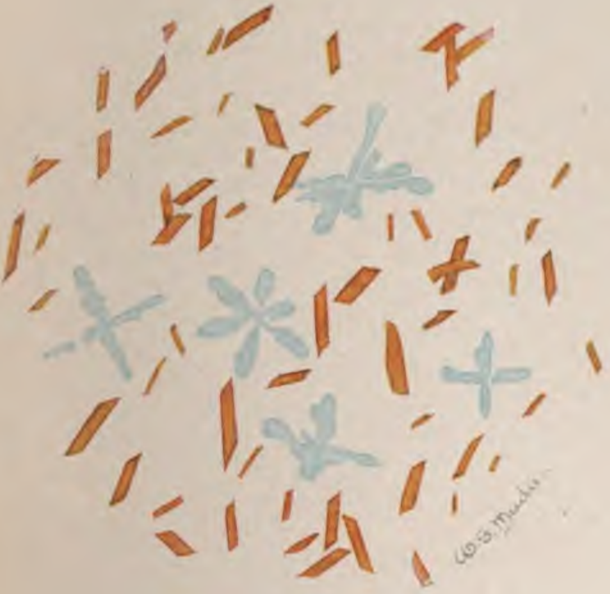
CHAPTER I.

THE BLOOD.

It is our purpose in this chapter to describe briefly the physiology of the blood, and to give in detail the technic of available methods for its recognition and clinical study, by which methods we may gain diagnostic and prognostic knowledge. Included will be found remarks upon the blood-changes induced by disease. This subject will be taken up under the following heads:

(*a*) Tests for the recognition of blood.
(*b*) Estimation of the total volume of blood and of its oxygen-carrying capacity.
(*c*) Study of fresh blood:
    1. Microscopic study both with and without the warm stage.
    2. Estimation of the relation of the volume of the corpuscles to that of plasma.
    3. Ascertainment of the specific gravity of the blood.
    4. Estimation of the time necessary for coagulation.
    5. Estimation of the amount of coloring-matter and of the blood-pigments.
    6. Counting of the red and white corpuscles.
    7. Reaction of the blood.
(*d*) Composition of the whole blood.
(*e*) Ascertainment of the freezing-point (cryoscopy).
(*f*) The osmotic properties of the blood.
(*g*) Study of smears, dried and stained.
(*h*) Bacteriologic examination of the blood.
(*i*) Abnormalities of the serum and serum-diagnosis.
(*j*) Special pathology of the blood.
(*k*) Parasitology of the blood.

PLATE I.



HEMIN CRYSTALS.

TESTS FOR THE RECOGNITION OF BLOOD.

The most delicate and decisive test for blood is the demonstration of the red blood-cells under the microscope, and next in order should be classed spectroscopic study (see *Hemoglobin*, page 41).

Teichmann's Test.—Collect a small portion of the suspected substance or débris, and place upon the center of a slide; add a few drops of salt solution, macerate, and mix well. Evaporate the mixture to dryness, moisten the residue with glacial acetic acid, and apply a cover-glass. Heat one end of the slide gently over a flame (avoid boiling). While the slide is being heated, allow a drop of glacial acetic acid to run in from the edge of the cover-glass from time to time, cool, and examine under the microscope for hemin crystals (Plate 1).

Caution.—Excessive heat prevents the formation of hemin crystals by driving off free HCl. Too much heat also produces alterations in the albumins which may retard and at times prevent the formation of crystals.

Guaiacum Test.—Prepare an aqueous solution of the suspected substance and to it add a few drops of tincture of guaiacum, which gives it a milky precipitate. Add a few drops of hydrogen peroxid (or Merck's oil of turpentine)—in the presence of blood-pigment a distinct blue color is immediately produced.

Caution.—The tincture of guaiacum should be freshly made and diluted to the color of sherry wine. This test is capable of demonstrating one part of fresh blood in several thousand parts of water, and may be employed in the study of feces, p. 360.

ESTIMATION OF THE TOTAL VOLUME OF THE BLOOD.

The limits of error in the various methods for the total estimation of the blood are equally great with those of the physiologic and pathologic variations, therefore it is questionable whether any known methods are of great clinical value. Haldane and Smith, however, have suggested a method which bids fair to stand the test of criticism.

Haldane and Smith Method.—(*a*) The patient is permitted to inhale a measured volume of CO, and after three minutes a few drops of his blood are withdrawn for analysis, and the degree to which the hemoglobin is found to be saturated with this gas is then estimated.*

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*"Jour. of Physiology," vol. xxii, p. 232; also vol. xxv, p. 331.

(*b*) The degree to which the blood has been saturated by CO is now known, and the quantity of this gas which may be taken by the whole of the patient's blood is then estimated. Let us suppose that 150 c.c. of carbonic oxid has been administered, and that the blood is found saturated to the extent of 25 per cent., in which case it is readily seen that 600 c.c. would have been required had the blood been completely saturated, or the total capacity for CO and likewise for oxygen is 600 c.c.

(*c*) In order to estimate the oxygen capacity per 100 c.c. in any given sample of blood accurately, its color is compared with an equal volume of ox blood whose oxygen capacity has been estimated; *e. g.*, after the patient has absorbed 100 c.c. of CO his blood is found to be one-fifth saturated by this gas; therefore the total capacity for CO or for oxygen is 500 c.c. Furthermore, this patient's blood is found to present the same color as that of ox blood, of which every 100 c.c. has been found capable of taking up 20 c.c. of oxygen. This patient's total oxygen capacity, 500 c.c., when divided by the oxygen capacity of every 100 c.c., gives the patient's total oxygen capacity (2500 c.c.); we divide this sum by the oxygen capacity of 100 c.c. of his blood, which is 20, and derive 25. This figure 25 is the factor for the number of cubic centimeters of blood in the body (2500).

Smith, as a result of a series of estimates, gives the average in health as 3420 gm. In the normal cases studied he found this figure to vary from 2830 to 4550 gm., or in a ratio of one-thirtieth to one-sixteenth of the body-weight.

STUDY OF THE FRESH BLOOD.

PREPARATION OF SLIDES AND COVER-GLASSES.

Both slides and cover-glasses should be cleansed for use in the following manner: Place a number of cover-glasses or slides in a glass containing warm water and soap, when they should be stirred with a glass rod, after which each cover or slide is removed separately, placed upon a thin handkerchief, and rubbed gently between the thumb and forefinger. After it is thoroughly dried, it should be dropped into a second glass containing warm water. After all the covers have been transferred in this manner from the soap and water to the second glass, they are again stirred by the glass rod, and, receiving the same manner of treatment, are transferred to a third glass, which contains 70 per cent. alcohol. The treatment in the alcohol should be the same as above outlined, after which these cover-glasses are placed in a wide-mouthed bottle containing equal parts of alcohol and ether, in which solution they are kept for use.

Caution.—All cover-glasses and slides should be removed from the solution in which they are kept by means of clean forceps. They should then be dried with a soft linen or silk handkerchief, and placed separately in a Petri dish, the bottom of which has been neatly covered with a piece of filter-paper. After the desired number of slides or covers have been removed, the dish is covered and set on a warm stage or in an incubator, in order that the slides may be warm when the blood is applied—a condition which materially facilitates securing an equal distribution throughout the smear.

Clinical Blood Reports.—The following is a copy of the blank used by me for clinical blood reports in the Clinical Laboratory of the Medico-Chirurgical College, Philadelphia:

Name:
Specimen Received at Laboratory: Mo. Day Hour .M.
Date of Examination:

BLOOD EXAMINATION.

Percentage of Hemoglobin:
Color-index:
Number of Red Corpuscles in 1 c.mm.:
Description:

Nucleated Erythrocytes: { Normoblasts
Microblasts
Megaloblasts
Number of Leukocytes in 1 c.mm.:
Differential Count Based on Percentage in Corpuscles:
Small Lymphocytes
Large Lymphocytes
Polymorphonuclear Neutrophils
Transitional Forms
Eosinophils
Basophils
Myelocytes
Eosinophilic Myelocyte
Other Forms

Plasmodia of Malaria:
Widal Typhoid Reaction:
Other Serum Reactions:
Bacteria Found (by Spreads or by Cultures):
Alkalinity:
Blood-plates in 1 c.mm.:
Coagulation Time:

COLLECTION OF BLOOD.

The points of election for the collection of blood are the lobe of the ear and the under surface of the finger near its tip; and the method to be described is employed in all clinical work except bacteriologic study (see *Bacteriology of Blood*, page 109).

The first step in the collection of blood is made as follows: Should the tip of the finger be chosen, cleanse the tip with water, rub gently with a soft cloth, and later moisten with ether. After the ether has evaporated, the finger is grasped between the thumb and index-finger of the left hand, and the instrument with which the puncture is to be made, having first been cleansed with water and alcohol, is quickly plunged into the tip. The depth of this puncture should vary with the thickness of the skin (one-eighth to one-fourth of an inch). Gentle pressure may be applied to start the blood flowing, after which four or five drops of the first blood exuded should be removed with a soft handkerchief. When the ear is chosen, the same precautions are to be observed. The puncture may be made with Daland's blood-lancet (Fig. 13), which it has been my practice to employ, or with a new steel pen after one nib has been broken away. A ***round pointed needle should not be employed.***

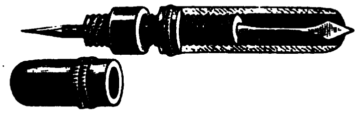

Fig. 13.—Daland's blood-lancet.

Caution.—Avoid pressure, since it causes a diminished number of the cellular elements to exude in connection with a large amount of serum.

IMMEDIATE EXAMINATION OF BLOOD AND MAKING OF SMEARS.

Immediate Examination.—After the first four or five drops of blood have been removed by the handkerchief and a rather large-sized drop exudes spontaneously, the cover-glass (Fig. 14) (which has been warmed) is held by its edge between the thumb and index-finger, and the summit of the drop of blood is allowed to touch the center of the cover-glass, care being taken that the surface of the glass does not come in contact with the skin. The cover-glass is allowed to fall gently upon the center of a slide (Fig. 15), the weight of the cover-glass causing the blood to spread between it and the slide.

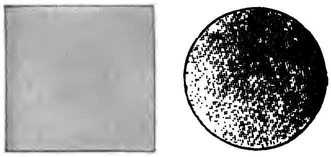

Fig. 14.—Styles of cover-glasses.

Caution.—When pressure is used to spread the blood, reliable deductions cannot be drawn from the microscopic appearance of such specimens, since pressure is liable to cause distortion of the cellular elements. The slide may now be carried to the microscope and examined by any power objective desired.

Smears.—When smears are to be made, the above process is modified only in that the cover-glass with its specimen of blood is allowed to fall upon another cover-glass in such a manner that the margin of the one projects beyond that of the other, and after the blood has spread, these overlapping margins are grasped between the thumb and index-finger of each hand and separated by pulling on the horizontal (Fig. 16). They are then laid upon a flat surface, specimen up, and allowed to dry in the air; after which they may be placed together and kept for an indefinite period without further treatment.

Fig. 15.—Glass slides (natural size).

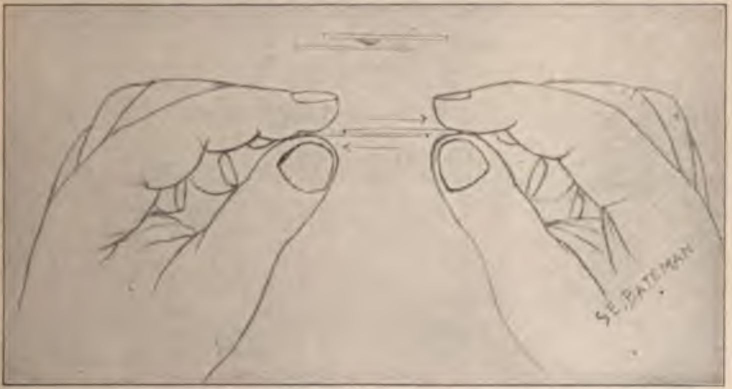

Fig. 16.—Cover-glass is touched to summit of a drop of blood and then allowed to fall on a second cover-glass, margins overlapping; method of separating cover-glasses.

A method which I have found more satisfactory for the beginner and for class work is to make the smears upon slides. The slide upon which the smear is to be made is laid upon the table, and a second slide is grasped between the thumb and index-finger, and one edge of this slide is brought in contact with the summit of the drop of blood in such a manner that the blood collects on its under surface and edge. The second slide is now placed at right angles to the first (which rests upon

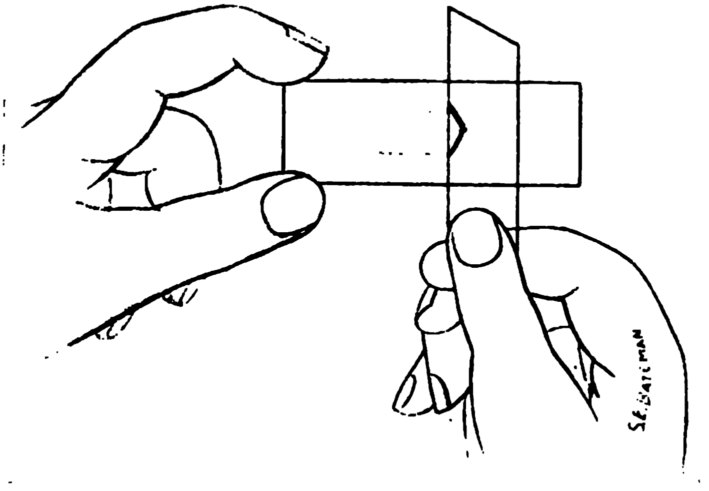

Fig. 17.—Method of smearing fresh blood on the slide.

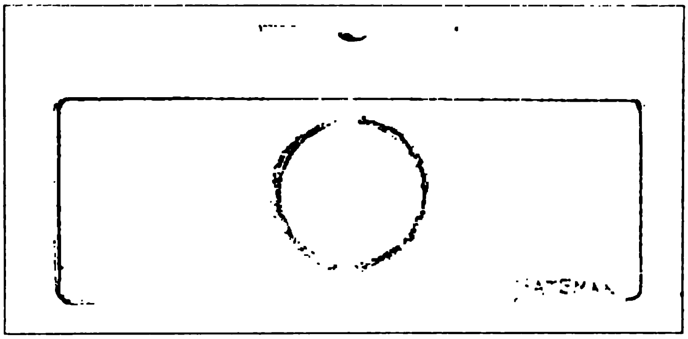

Fig. 18.—Semicircles of cedar oil or of vaselin at center of slide; cover-glass from which drop of blood is suspended

the table) and so tilted as not to catch the corpuscles between the edge of the second and the surface of the first slide (Fig. 17); then it is forcibly drawn over the surface of the slide for its entire length. The object is to transfer the drop of blood collected on the edge of the second slide to the surface of the first slide by allowing it to trail. It requires far less skill to make good spreads by this method than by the use of cover-glasses.

Life of the Blood-cells.—Death of the cells may not occur for several hours when the cover-glass and specimen have been placed upon a slide as previously described; but when it is desired that the cell's death be deferred further, the following method in addition to preserving life, prevents coagulation as well as distortion of the cells over a much longer period. Estimating approximately the size of the cover-glass to be used, an incomplete narrow band of cedar oil or vaselin is made upon the center of the slide, the oil being distributed to form two semicircles whose diameters are separated for a distance of one-eighth of an inch (Fig. 18). The cover-glass containing the blood is now allowed to fall, specimen down, upon the oil, when by its weight the oil from the two hemispheres is forced to unite, forming a complete ring, hermetically sealing the specimen.

Caution.—A complete ring of vaselin causes more air to be retained with the specimen and thereby favors destructive changes.

In studying the various animal parasites of the blood this method is most satisfactory, and is also useful in studying rouleaux formation. The filaria and the malarial organism may in this way be kept alive for a long time.

MICROSCOPIC STUDY OF FRESH BLOOD.

Fresh blood collected for study in either of the methods previously described may be placed on a warm stage or in an incubator; or, better still, upon the microscope, which is placed with its specimen in a warm oven and removed from time to time for examination.

The character of the blood as it exudes from the incision is worthy of special consideration, since it flows more readily than usual in such conditions as peripheral congestion, vasomotor dilatation, decided fluidity of the blood, chlorosis, certain anemias, and hemophilia. Blood usually flows sluggishly in such conditions as extreme anemia (primary or secondary), after hemorrhage, diarrhea, cholera, exposure to cold, hysteria, uremia, vasomotor spasm, and in increased coagulability.

Microscopically we may detect the Plasmodium malariæ, Filaria sanguinis hominis, spirochæte of relapsing fever, trypanosoma, Piroplasma hominis, and rouleaux formation. Again, but with far less accuracy, we may determine whether the blood contains an excess of fibrin, whether there is decided anemia, leukocytosis, or a deficiency or excess of hemoglobin; we may make an approximate estimation of blood-plates, and determine whether the red cells display any abnormality as to size and form. It requires a thoroughly skilled microscopist to detect with certainty the existence of these latter named conditions, and perfection in this particular can be attained only by actual experience. The ameboid movements of the leukocytes and the malarial parasite, the irregular contraction of dying protoplasm, which causes false motions in certain of the crenated points of normal red cells, and the Brownian movement of the protoplasm of corpuscles, must be carefully distinguished. Language seems inadequate to describe these differences definitely.

ESTIMATION OF THE VOLUME OF THE RED CELLS AND THE PLASMA.

This is accomplished by means of the **hematocrit,** which was first suggested by Blix, and has undergone several modifications; until the present instrument, as suggested by Judson Daland, appears to meet the demands necessary for clinical investigation. Its direct object is to ascertain the relative volume of the corpuscles and of the plasma in a given quantity of blood. Many of its advocates, however, contend that it will replace the Thoma-Zeiss instrument in the estimation of the red cells, but as yet it has not displaced this instrument in either foreign or American laboratories. It must be admitted that Daland's claims for the instrument are correct, and that for clinical purposes the red cells can be closely estimated with sufficient accuracy, except in cases where a high grade of leukocytosis exists (leukemia).

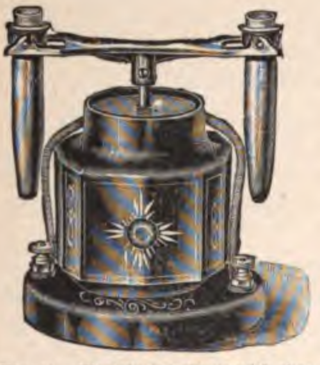
Fig. 19.—Electric centrifuge with rheostat.

The improved electric centrifuge consists of an iron-clad motor (Fig. 19) carrying an armature or horizontal bar, which may be replaced by the bar of the hematocrit (Fig. 20). A speed indicator should also be attached which strikes a bell every 100 revolutions, and a rheostat employed to control the current and speed. A speed of 8000 to 10,000 revolutions may be obtained by the street-current or from a small battery. The centrifugalizing should be continued for from two to four minutes. Less satisfactory results are to be gained by the employment of the hand centrifuge

(Fig. 21). The hematocrit armature contains two capillary tubes, which are held in position by springs (Fig. 20).

Process.—When it is possible for the patient to come to the laboratory, fresh blood is used. The capillary tubes are removed from the armature and filled by holding the tube horizontally and touching its tip to the summit of a rather large drop of blood. It is then inserted into the armature as seen in figure 20, the opposite tube filled with water, and the centrifugalizing begun before the blood has had time to coagulate.

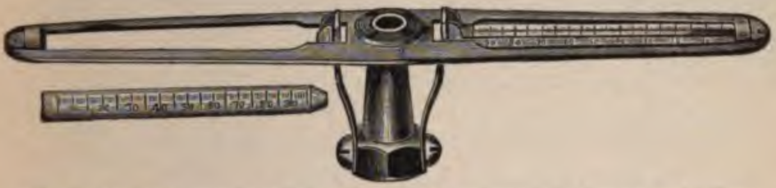

Fig. 20.—Daland's armature of centrifugal machine (hematocrit). One tube in position.

In estimating the blood of patients situated at a distance it is necessary to dilute the blood in a 2.5 per cent. solution of potassium bichromate (Daland). A dilution to one-half with this solution is accomplished by using either the white cell or the red cell pipet of the Thoma-Zeiss hemocytometer. In this instance it may be well to fill both tubes of the hematocrit with diluted blood. Healthy blood after it has been centrifugalized for from two to four minutes will show a sediment registering at about 50 per cent. (graduation on the tube of the hematocrit). One per cent. represents about 1,000,000 red corpuscles, and, therefore, it is necessary to add five ciphers to the percentage volume found, thus obtaining the number of red corpuscles in 1 c.mm. of undiluted blood; *e. g.*, suppose the reading on the graduated tube (see Fig. 20) is 40, multiply this figure by 100,000, which equals 4,000,000, the number of red cells in 1 c.mm. of undiluted

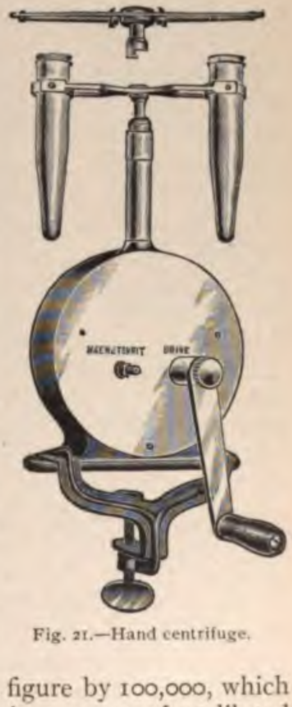

Fig. 21.—Hand centrifuge.

blood. The leukocytes often form a thin band at the central end of the column of red corpuscles and should not be included in the reading.

Daland in working with diluted blood concludes that the number of cells for each degree of the scale should be placed at 99,390—practically 100,000. When undiluted blood is used in the estimation, there appears to be greater liability to error. The size of the red cells differs widely in the various forms of anemia, and such differences must influence the readings obtained by the hematocrit.

For description of Wetherill's Torkrit see p. 523.

ESTIMATION OF THE SPECIFIC GRAVITY.

The most available method for clinical use is that suggested by Hammerschlag,* which is a modification of Roy's † method.

Hammerschlag's Method.—Two solutions (chloroform and benzol) are to be mixed in a urinometer glass in such proportions that the specific gravity, taken by an ordinary urinometer, is 1.059, or that of normal blood; the former of these solutions being heavier than blood, the latter lighter. The finger is punctured, a drop of blood is collected in a Thoma-Zeiss pipet, and a drop or two blown into the chloroform-benzol solution. The blood shows no tendency to mix with this solution, but floats as a ruby bead. Should the beads sink to the bottom, chloroform is added, and should they rise to the top, benzol is added drop by drop until the blood remains stationary in the body of the liquid. Since the specific gravity of the blood is that of its surrounding mixture, take the specific gravity of this liquid by means of an ordinary urinometer and the graduation figure obtained equals the specific gravity of the blood.

Caution.—Add chloroform or benzol a few drops at a time, stirring with a glass rod after each addition. Avoid having any air with the drop of blood. Rapidity is necessary; and a urinometer with a scale graduated to 1.070 is best for this purpose.

The specific gravity of the blood has been found to bear certain parallels with the percentage of hemoglobin, yet this may not be constant. The specific gravity of the plasma varies but little except in dropsy, and in the corpuscles themselves the variable element is the hemoglobin. In most non-dropsical patients the specific gravity of the whole blood bears a more or less direct relation to the hemoglobin. In leukemia the specific gravity is relatively higher than the hemoglobin, and is dependent upon the weight of the leukocytes; while in pernicious anemia, where a high color-index is to be found, the hemoglobin is slightly higher than one would gage it from the specific gravity. The accompanying table will enable one to estimate the value of a given specific gravity in per cent. of hemoglobin, according to Schmaltz:

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* "Wien. med. Wochensch.," 1890, vol. iii, p. 1018.

† "Proceedings Phys. Soc.," 1884.

Spec. Grav.		Hemoglobin.	Spec. Grav.		Hemoglobin.
1.030	=	20 per cent.	1.0455	=	50 per cent.
1.035	=	30 "	1.048	=	55 "
1.038	=	35 "	1.049	=	60 "
1.041	=	40 "	1.052	=	70 "
1.0425	=	45 "	1.056	=	80 "
1.059	=	100			

Wright's Coagulometer.—This instrument (Fig. 22) is composed of a central metal cylindric can, surrounding which is a closely fitting leather case containing nine small pockets, each lined with flannel. One of these pockets holds a thermometer graduated to 50° C. (Fig. 22). The remaining pockets serve as receptacles for the capillary blood pipets. Each pipet is composed of clear glass, is about 10 cm. in length, and has a lumen of 0.25 mm.

Method.—1. Fill the cylinder with water at the body-temperature (37° C.—$98\frac{3}{5}$° F.).

2. Label the tubes a, b, c, etc., and place them in the pockets, and when they are warmed, obtain a rather large drop of blood from the tip of the finger and from it fill, by aspiration, tube (a) to one-half its capacity and immediately place this tube in its pocket.

Caution.—Note the time of filling tube labeled (a) and also record the condition of its blood when examined three minutes later.

3. In a like manner several of the tubes are filled with blood at intervals of one minute.

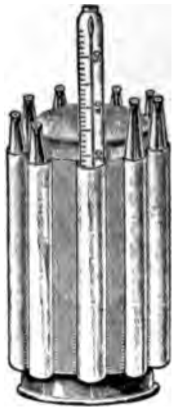
Fig. 22.—Wright's coagulometer.

4. Remove the tubes from the instrument at different lengths of time after the blood has been withdrawn, and endeavor to force the blood from the tube by blowing. The time necessary for the blood to clot in the tube is regarded as the *coagulation time*.

Cleansing the tubes is accomplished by passing a fine wire into the tube and breaking the clot, after which the tubes are washed with water, alcohol, and lastly with ether.

For description of Biff's Hemogelometer see p. 523.

HEMOGLOBIN.

This proteid contains nearly 96 per cent. of albumin and 4 per cent. of pigment (hemochromogen). In the red cells hemoglobin probably exists in combination with the nucleoproteid of the stroma. The characteristics of this union are not understood, yet it certainly renders hemoglobin practically insoluble, concentrated, and capable of forming with great rapidity indefinite compounds with oxygen. The chemic composition of hemoglobin is both complex and variable. Its spectroscopic relations, however, are constant; and while in the circulation it exists in the veins principally as reduced hemoglobin; and in the arteries, on account of molecular union with oxygen, as oxyhemoglobin. Each gram of saturated oxyhemoglobin contains 1.16 c.c. of oxygen, but this degree of saturation varies greatly. Blood titrated with a standard solution of tartaric acid does not give the characteristic absorption-bands of oxyhemoglobin, methemoglobin, and carbon-monoxid hemoglobin after the alkalinity is neutralized (Dare).

Oxyhemoglobin is non-diffusible, of a bright red color, and while it crystallizes with difficulty, may form yellowish-red rhombic plates which are readily soluble in water and in weak solutions of the alkaline carbonates. They are insoluble in strong alcohol, ether, carbon disulphid, benzol, and chloroform. Labbe * found an increased quantity of oxyhemoglobin in the new-born, ranging from 15 to 16 per cent. During the first ten days of extra-uterine life it falls to 14 per cent. Such bloods likewise contain a high percentage of reduced hemoglobin.

Reduced hemoglobin is of a dark cherry-red color, but after high dilution it may display a greenish tint. It is not readily crystallized, but is more freely soluble than is oxyhemoglobin. It is demonstrable in the blood of asphyxia and in the new-born.

Hemoglobinemia (hemocytolysis) is a condition characterized by a solution of the hemoglobin in the plasma; and in man is a pathologic condition which probably results from lowered vitality of the erythrocytes, and also from abnormalities in the plasma (see *Viscosity*, page 88). Diminished resistance of the red cells may be found to accompany the hemoglobinemia following extensive burns (see *Isotonic Tension*, page 73), while the hemoglobinemia seen after poisoning may, in part at least, be due to changes in the serum.

Methemoglobin displays a brownish-red color, and crystallizes as brownish-red needles, prisms, and hexagonal plates. It is readily soluble in water and contains about the same proportion of oxygen as oxyhemoglobin, but the oxygen appears to have formed a rather firm union. It is observed in connection with poisoning.

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* "Revue de médecine," Dec. 10, 1900 (20me Année, No. 12).

Carbonic-oxid hemoglobin is the name given to hemoglobin containing CO, which gives the blood a rose-red or bluish color. Its crystals display a slight bluish tint, and are not easily dissolved. It is met with after poisoning by the inhalation of illuminating gas, and may remain in the blood for a period of several days.

Hematin (Plate 1) appears in the feces after gastro-intestinal hemorrhage, in bloody transudates and effusions, and in the urine after poisoning with arsenic.

Hematoidin is a derivative of hemoglobin, and appears either as needles or rhombic plates which are of a light- or dark-orange hue, soluble in ether, carbon disulphid, ammonium disulphid, and chloroform. It absorbs most of the violet end of the spectrum, but does not contain iron (Fig. 24).

Hematoidin occurs in bloody exudates of long standing and in the urine after traumatism to the kidney (Yarrow). (See Plate 17.)

Hemosiderin results from the destruction of hemoglobin; is amorphous, and occurs in the viscera after extensive blood destruction.

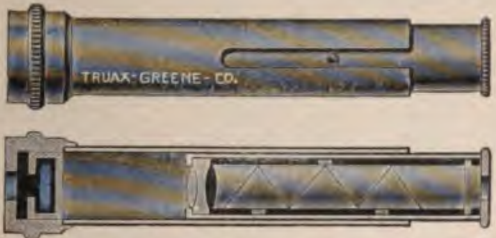

Fig. 23.—Browning's spectroscope.

Melanin is a yellowish-brown or black pigment, insoluble in water, alcohol, chloroform, ether, and weak acids. It is soluble in strong alkalis, destroyed by heat, and does not give reactions for iron. It results from the action of the malarial parasite upon the hemoglobin, and should be distinguished from other pigments which may or may not contain iron, and whose origins are unknown.

Spectroscopic Examination of the Blood.—Spectroscopic examination of the blood serves as the most reliable test for the recognition of blood pigments, and also for the determination of the particular form of pigment. A one per cent. solution of fresh blood is found to produce distinct absorption-bands, and when the blood has become dried, it is necessary to dissolve it by macerating in acetic acid. With such blood the spectrum of acid hematin is obtained. Blood from recent clots may be dissolved in water. When heat has been applied to the blood, macerate in a solution of ammonia, when the spectrum of reduced or alkaline hematin appears (Fig. 24). Browning's spectroscope (Fig. 23) will be found satisfactory when strong daylight or gaslight is employed. A collar serves to enlarge or diminish the aperture, and will be found necessary in different strengths of light; also when fluids of different opacities are used. Fraunhofer's lines are brought into focus by careful adjustment of the tube. Fluid to be examined should be placed in small glass vials with flattened surfaces. The spectrum of fresh arterial blood is that of *oxyhemoglobin,* and shows two absorption-bands between D and E (Fig. 24): one of these is sharp, dark, and well-defined near the orange, D; the other is wider, less sharply defined, and rests near the green, E. The indigo and most of the blue will be absorbed, and in strong solutions of oxyhemoglobin these two bands may unite.

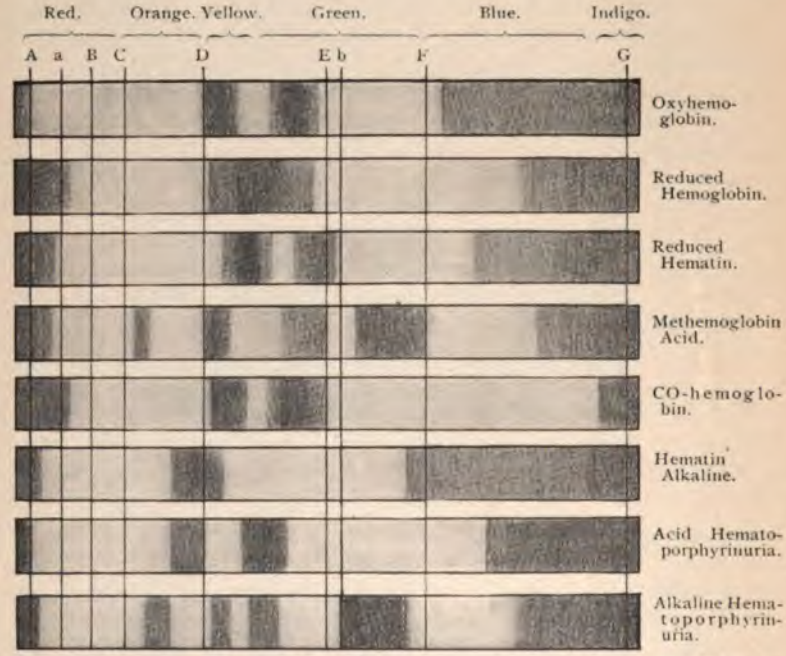

Fig. 24.—Diagram of the spectra of eight substances known to concern us from a diagnostic standpoint.

In case ammonium sulphid is added to such solutions, the color of the fluid becomes dark, and the spectrum changes to that of *reduced hemoglobin*, when one band of absorption occurs between D and E (Fig. 24). A positive indication of the presence of blood is that the spectrum may be transformed from that of oxyhemoglobin to that of reduced hemoglobin by the addition of reducing agents to the solution. Cochineal and ammoniated carmin give spectra simulating that of oxyhemoglobin. The addition of boric acid to a solution of these substances causes their spectra to be displayed by the blue, while the spectrum of blood is unchanged. Other of the vegetable dyes have spectra simulating those of blood, but these become pale upon adding sodium bisulphite.

Methemoglobin.—Hematin is produced by adding acids or strong alkalis to reduced hemoglobin. In acid solution its spectrum simulates that of acid methemoglobin, while in alkaline solution it gives a broad band at D (Fig. 24). A clinically important change is that of oxyhemoglobin into methemoglobin, which is detected by the chocolate color of the blood, and in acid or neutral solution it gives four absorption-bands: one rather distinct between C and D; a second faint narrow band in the yellow just to the right of D; a third broad, fairly distinct band between the yellow and the green just to the left of E; and a fourth broad band to the left of F, which sometimes extends beyond the line F into the blue.

Carbonic-oxid-hemoglobin is detected in cases of poisoning by illuminating gas, and to the naked eye is of a rose-red color in both arterial and venous blood. A one-half of one per cent. dilution of such blood gives a spectrum that differs from that of oxyhemoglobin only in that its bands are broader and that the band at D is displaced to the right. The addition of ammonium sulphid causes the spectrum of oxyhemoglobin to be replaced by that of reduced hemoglobin, while that of carbonic-oxid-hemoglobin is unchanged.

Corroborative Tests.—Prepare a two per cent. solution of blood and to it add a few drops of orange-colored ammonium sulphid containing an excess of sulphur; add a few drops of dilute acetic acid, and shake gently to effect a mixture. Carbonic-oxid blood is recognized by displaying a rose-red color, and disseminated throughout the mixture are seen flocculi; while normal blood assumes a greenish or reddish-gray color. This test may be accomplished in a test-tube. A dilute solution of zinc chlorid or of platinum chlorid gives a bright-red color with carbonic-oxid blood, while normal blood is changed to black. By diluting the blood solution four or five times with acetate of lead, carbonic-oxid blood changes to red, while normal blood becomes chocolate. Diluting a neutral solution of carbonic-oxid hemoglobin, a clear red coagulum results; while oxyhemoglobin turns grayish-brown. Warming the blood with an equal amount of a 10 per cent. NaOH solution, normal blood becomes a dark brownish green; while carbonic-oxid blood is first cloudy, later clear red, and red flocculi gather on the surface.

Dare's Hemoglobinometer.—This instrument was devised by Arthur Dare, of Philadelphia, for the purpose of registering the percentage of hemoglobin in the undiluted blood. For colorimetric observations with this instrument the drop of blood is collected by capillary attraction into a chamber of definite thickness and of sufficient surface for a color field. Illumination is effected by candle light. Between the stratum of blood and the flame of the candle a white glass slide is interposed to diffuse the direct rays of light. The color of the blood is compared with a graduated color prism—a tinted glass which represents the hemoglobin in the blood examined. The blood pipet (Fig. 25, D, A, B) when filled is placed into a special receptacle upon the side of the instrument and directly between the eye-piece and the flame of the candle. After the candle flame (Y) and the capillary pipet (W) are properly adjusted, the eye-piece (U), which is telescopic, is adjusted

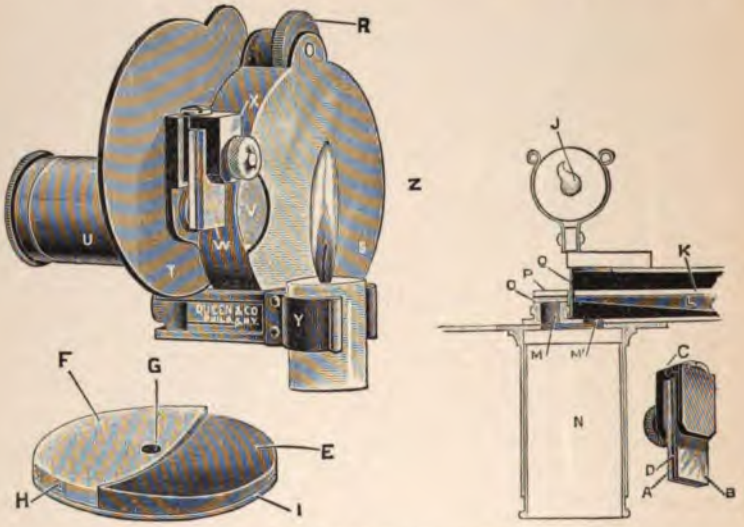

Fig. 25.—Dare's hemoglobinometer.

to accommodate the observer. The color prism is changed by using the milled wheel (R) at the top of the instrument. By this instrument the hemoglobin is estimated in a much shorter time than can be done by any other hemoglobinometer, and after one has become thoroughly proficient in reading color with one eye closed, and accustomed to the necessary variations, I see no reason why Dare's instrument should not be considered a valuable addition to our clinical laboratory apparatus.

I have studied the blood from a large number of patients from the Medico-Chirurgical Hospital Dispensaries, correlatively using this instrument with that of von Fleischl and of Oliver, and while the readings obtained by Dare's instrument have not at all times agreed with those obtained by the other instruments, its registration has always been sufficiently close to enable me to recommend it to the profession. Contrary to the statements commonly heard concerning Dare's instrument, my experience shows that it requires more time and attention to detail to teach the student to estimate hemoglobin with this instrument than with the other instruments considered. From ten to twelve seconds should be the time for observation.

Oliver's Hemoglobinometer.—Oliver's instrument enables us to estimate more closely the percentage of hemoglobin than has hitherto been accomplished by other instruments devised for this purpose. It consists of a series of 12 stained-glass discs, corresponding to the hemoglobin percentages from 10 to 120, which are arranged in two rows (Fig. 26). This instrument as used for general clinical work has two riders, a lighter and a darker. Intermediate shades are measured by means of colored glass riders which are placed on top of the primary color disc to deepen the tint seen, and make it correspond to that of the graduated colored disc. A capillary pipet (Fig. 33, *a*) is used to collect the blood, which is washed into the mixing cell (Fig. 26, *e*) by means of a medicine dropper provided with a rubber tube to fit over the blunt end of the pipet. The mixing cell is filled to the brim with distilled water and covered with a small glass plate. The bubble which forms underneath this plate should be small, and the cell so turned as to prevent any shadow upon the color field. The cell is now brought close to the scale and compared with the tints of the different standard discs. Should it match one of these, the observation is complete. Should it not, one of the glass riders, capable of reading within 2½ degrees, is placed upon the chamber, and the particular disc it matches noted. It may be necessary to apply more than one rider, and if desired riders may be obtained which register a single degree. The standard was originally arranged for candle light, and discs standardized for reading by daylight are seldom used. The outer light is excluded by the use of a hydroscope tube; the matching of colors is quite simple and does not require special description.

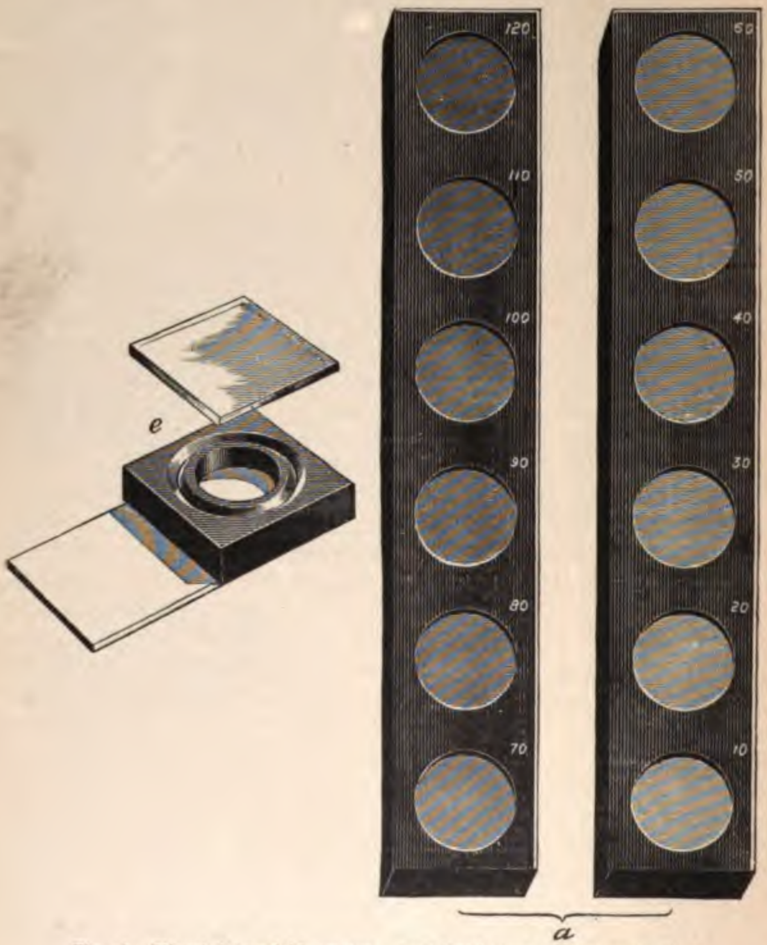

Fig. 26.—Oliver's hemoglobinometer: *a*, Colored discs; *e*, mixing chamber.

Von Fleischl's Hemoglobinometer.—This instrument consists of a stand which somewhat resembles the base and table of a microscope; a ground cylinder, which is divided into halves; a plaster-of-Paris reflector; a graduated color prism; a thumbscrew adjustment; and a capillary tube (Fig. 27).

Method of Application.—1. One compartment of the graduated cylinder is half filled with distilled water, care being previously exercised to cleanse the chambers of the cylinder.

2. The capillary tube, which has been previously cleansed and dried, is held upon the horizontal and brought into contact with the summit of a drop of blood. It fills immediately by capillary attraction. All blood clinging to the outer surface of the tube is removed by a soft handkerchief, and the tube immersed in the distilled water contained in the cylinder. It is held horizontally, shaken gently until apparently emptied, when a few drops of distilled water are forced from a dropper through the tube in order to wash every particle of blood into the cylinder.

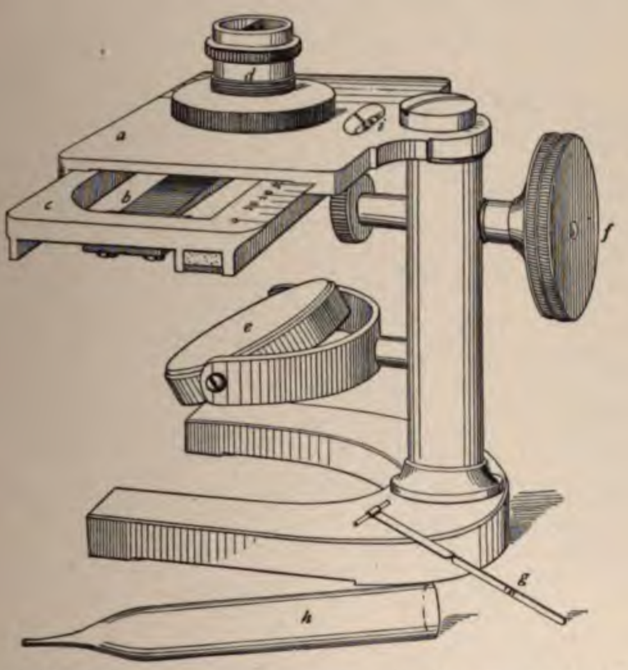

Fig. 27.—Von Fleischl's hemoglobinometer: *a*, Stand; *b*, narrow wedge-shaped piece of colored glass fitted into a frame (*c*), which passes under the chamber; *d*, hollow metal cylinder, divided into two compartments, which holds the blood and water; *e*, plaster-of-Paris plate from which the light is reflected through the chamber; *f*, screw by which the frame containing the graduated colored glass is moved; *g*, capillary tube to collect the blood; *h*, pipet for adding the water; *i*, opening through which may be seen the scale indicating percentage of hemoglobin.

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3. After the expulsion of the blood stir the solution gently with the metal handle of the pipet to effect a perfect mixture.

4. By the use of a dropper fill both compartments of the cylinder to the brim with distilled water. Moisten with the breath the ground cover-glass, and then allow it to fall gently upon the cylinder, when there will appear two small bubbles, one upon each side of the partition. Should the bubble upon the side containing the blood be large, it is evident that sufficient diluting fluid has not been added and that our reading will be too high. When too large an amount of distilled water has been added, the solution containing the blood may be forced across the partition to mingle with the clear water on the other side, this again defeating our purpose.

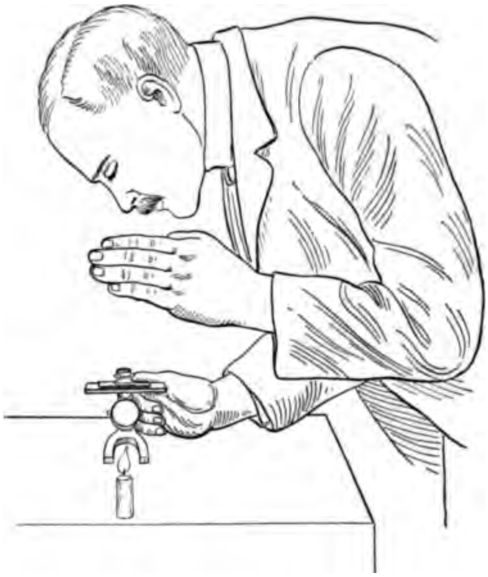

Fig. 28.—Estimation of hemoglobin by von Fleischl's instrument, showing position of instrument, candle, and operator. Left eye closed, reading with the right only.

5. With the cover-glass properly adjusted, turn the cylinder so that the portion containing the blood will be toward the light; thus the portion of the cylinder containing only distilled water is over the graduated color prism.

6. A dark room is necessary in this estimation, and the candle should be placed directly in front of the instrument (Fig. 28),

and the plaster-of-Paris mirror so adjusted as to bring the column of light through the solution.

7. The operator should stand at the side of the instrument, bringing his face directly over it, and should read with the eye that is farthest from the light (Fig. 28) (all direct light from the candle should be obstructed from the eye by holding some object to the anterior edge of the instrument). This may be accomplished by rolling a paper in the form of a tube. I have not been able to acquire satisfactory results by reading with both eyes.

8. Use the thumb-screw with rather short and quick turns, since it is difficult to detect the changes in the color of the prisms when it is slowly moved.

9. When the percentage of coloring-matter is suspected of being extremely low, two or more pipetfuls of blood should be placed in the solution. It is impossible to estimate with any degree of accuracy when the reading is below 40 per cent. In teaching I have found that in a section of twelve students there is seldom a difference of twelve points (12 per cent.) in the readings they make after they have been privileged to use this instrument the third time. The wide scope of errors so commonly observed by others has not been my experience in using von Fleischl's instrument.

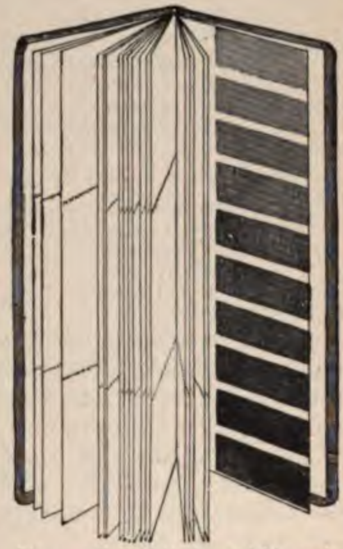
Fig. 29.—Tallquist's hemoglobin scale.

Tallquist's Hemoglobin Scale.—Tallquist has devised a color scale which is accompanied by a booklet containing small sheets of a special paper. The color scale is graduated from 10 to 100. Touch the summit of a rather large drop of blood with the paper, and as soon as the blood has been distributed over that portion of the paper which it will occupy the paper is laid beside the color scale, and moved to a point where it matches one of the color blocks (Fig. 29). I have found this method fairly satisfactory for the average clinical work, and am of the opinion that the person with limited experience obtains better results by this simple method than by those more elaborate methods previously described. For description of Wetherill's color tests see p. 524.

COUNTING OF THE BLOOD-CORPUSCLES.

Method of Thoma-Zeiss.—Among the many instruments devised for this purpose that of Thoma-Zeiss with possibly Zeppert's modification of the ruling of its counting chamber is doubtless the best. The use of the Thoma-Zeiss instrument is executed in the following manner:

1. The cleansing of the part for the puncture and the securing of a proper drop of blood are obtained as previously described (page 34). In addition it is necessary that the blood be diluted for this purpose and one of the following solutions is to be employed:

(*a*) Hayem's solution:

Mercury bichlorid	0.5	gm.
Sodium sulphate	5.0	"
Sodium chlorid	2.0	"
Distilled water	200.0	c.c.

(*b*) Toisson's mixture:

Methyl-violet	0.025	gm.
Sodium sulphate	8.0	"
Sodium chlorid	1.0	"
Pure glycerin	30.0	c.c.
Distilled water	160.0	"

These solutions will preserve the red cells for twenty-four hours, and their specific gravities are such that the cells precipitate slowly. Toisson's solution stains the leukocytes a violet tint. These solutions keep well, but should be filtered whenever they display the slightest clouding. A one-half of one to two per cent. solution of acetic acid is used as a diluent when we desire to count the white cells. It is to be remembered that acetic acid decolorizes the red cells.

The pipet for the counting of the red cells consists of a glass rod with an expanded portion near one end which contains a glass ball. Below this expansion it is graduated in tenths to one, and above the expanded portion to 101 (Fig. 30). To one end of this tube is attached a piece of rubber tube which has a bone nipple at its other extremity.

The pipet for the estimation of the white cells differs only in that it is larger and is graduated in tenths to one, and then above the expanded portion to 11. It will be readily seen that after the pipet for the red cells be filled with blood to 1, and then the solution be added until it reaches 101, the diluting of the blood will be one part of blood in 99 parts of the diluent, since there are 100 parts of solution between 1 and 101 (see *Method of Filling Tubes*, Fig. 31). The rubber tubing is now removed and the one part in the graduated portion is blown out. I usually fill the tube with blood to 0.5 and add the solution to 101, which minus the 1 below the expansion gives a dilution of 1 in 200.

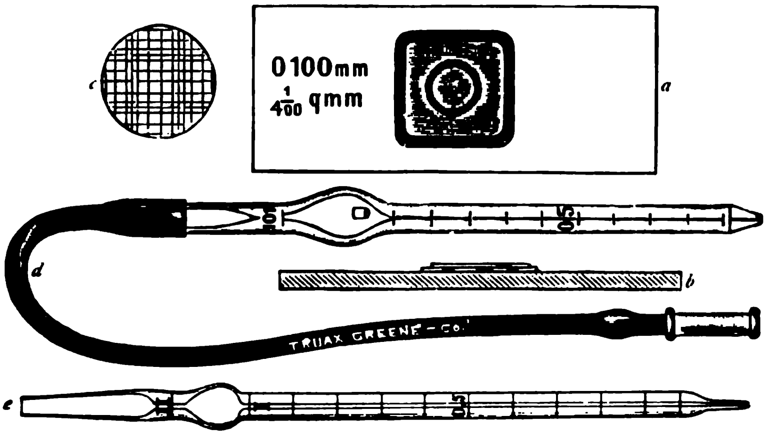

Fig. 30.—Thoma-Zeiss hemocytometer: *a*, Slide used in counting; *b*, sectional view; *c*, portion of ruled bottom of well; *d*, red pipet; *e*, white pipet.

The white pipet when filled to 1 with blood and the solution added to reach 11 gives us a dilution of 1 part of blood in 9 parts of the diluent, after forcing out the liquid in the capillary tube below 1. Here again I prefer to fill the tube with blood to the 0.5 graduation, and with the solution to 11, a dilution of 1 part

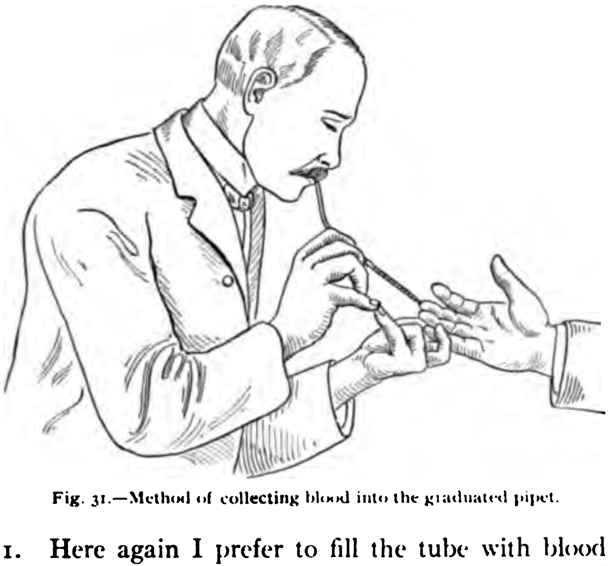

Fig. 31.—Method of collecting blood into the graduated pipet.

in 20. The diluting fluids should be carefully watched lest they become cloudy or contain any sediment which may obstruct the lumen of the capillary tube. To the summit of the drop of blood collected from either the ear or the finger the tip of the pipet is touched and gentle suction applied to the rubber tube, the nipple of which is placed between the lips (Fig. 31).

When the blood is drawn to the desired graduation, the tongue is placed against the opening of the nipple and the tube withdrawn from the drop, its tip cleansed with a soft towel, and the end of the tube now immersed in the diluting fluid. Suction is again made, and the pipet rotated rapidly between the thumb and index-finger as the diluting fluid enters (always holding the pipet in the vertical position). The glass ball in the expanded portion facilitates in effecting a dissemination of the blood throughout the diluting fluid; and when sufficient of the solution has been added to reach the 101 graduation, the tongue is again placed over the opening of the nipple, and the tube withdrawn from the solution. The rubber tube should be removed from the pipet, and the diluting fluid occupying the tube below its expanded portion should be blown out, since it plays no part in the dilution.

Caution.—In case the tube be held horizontally while filling it with the diluent, the solution is liable to creep along the side of the tube and pass the 101 graduation before the expanded portion is entirely filled, thereby greatly increasing the dilution and rendering worthless the results. Unless the rotation of the tube be rapid during its filling, the blood is liable to coagulate in the expanded portion or to be unequally distributed, when again the result obtained is of little value. Should suction be applied too vigorously, the liquid may be drawn along the side of the tube to beyond 101 before the expanded portion is filled.

If not avoided, these errors are responsible for more mistakes than any other steps in the estimation of either the red or the white corpuscles. After the tubes have been filled they may be placed together and secured by a rubber band. Unless the ends of the capillary tubes are permitted to touch some object, they will remain in perfect condition for hours, and further progress in the technic need not be continued immediately.

Counting.—Next in the process for the estimation of the blood-cells we have to consider the slide, which contains at its center a chamber divided into 400 squares, each of these squares being $\frac{1}{20}$ of a mm. square and $\frac{1}{10}$ of a mm. deep, and having a capacity of $\frac{1}{10} \times \frac{1}{20} \times \frac{1}{20} = \frac{1}{4000}$ of a mm. (Fig. 32). This chamber is surrounded by a narrow channel. Every sixteen of these small squares are surrounded by a double row of lines, and are known as a great square (Fig. 32, left corner).

There is a special ground cover-glass which must be brought in direct apposition with the slide in order that each of the squares be exactly $\frac{1}{10}$ of a mm. in depth. The tube containing the diluted blood is rotated rapidly between the thumb and finger. From four to eight drops of blood are forced out of the pipet by blowing through the rubber tube. A single drop is made to collect at the tip of the pipet and transferred to the center of the slide. Place the special cover-glass upon the edge of the raised portion of the slide. The forefingers are placed upon the cover-glass, while the second fingers and thumbs hold the slide at its corners. The forefingers are forced forward, using marked pressure until the cover-glass has passed beyond the opposite margin of the channel which surrounds the graduated chamber.

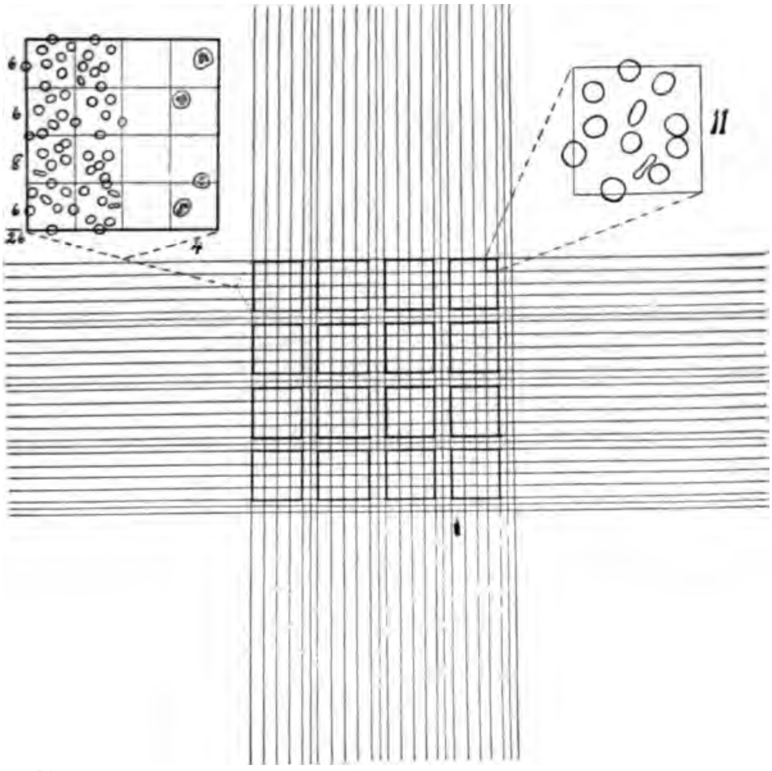

Fig. 32.—Thoma-Zeiss counting chamber. Capacity, $\frac{1}{400}$ mm. Sixteen great squares, heavily outlined, within the cross-lines, and bounded by double lines. Each great square contains 16 small squares. Each small square is $\frac{1}{10}$ mm. in depth by $\frac{1}{20}$ mm. square; $\frac{1}{10} \times \frac{1}{20} \times \frac{1}{20} = \frac{1}{4000}$ mm. Projected to upper left corner is one great square showing arrangement of red cells and number in each small square; also leukocytes in last column. Right hand shows small square containing 11 red cells.

The floor of the chamber is ruled into 400 small squares.

Should the cover-glass be in direct apposition with the glass shelf surrounding the chamber, there will be displayed at different points the colors of the rainbow (*Newton's rings*). Considerable skill is required in the application of the cover-glass. It may be permitted to drop gently upon the chamber, and should Newton's rings not appear, gentle pressure may be made with a needle, when they are likely to be seen; but should they disappear when the pressure is removed, it is evident that some dirt rests between the shelf and the cover-glass.

After the cover-glass has been successfully applied, the slide is placed upon the microscope, and should first be brought under the focus of a two-thirds objective, using a No. 1 or 2 eye-piece. If the corpuscles appear equally distributed throughout the field, a one-fifth or one-sixth objective is brought into focus. Either of these objectives will be found to include 16 of the small squares (one great square) (Fig. 32). In the counting of the red cells it is necessary to count the cells in five great squares (80 small squares). Since the factor to be employed in our estimation is extremely large, strict rules as to counting are necessary. Considering only the 16 squares surrounded by the double lines (Fig. 32), count the number of cells in each column of four squares.

Directions for Counting Red Cells.—In this estimation all corpuscles touching upon the top and left-hand boundary lines are included in the square, while those resting upon the right and bottom lines are to be included in the count of the contiguous squares (see Fig. 32, number of cells in each square and in column of squares being given). In this way it will be found easy to count the cells in the left-hand column and passing to the adjacent right-hand column until the four columns have been counted, which will give the total blood-cells for one great square (16 small squares). The slide is now moved and four other great squares are counted in like manner. A mechanical stage greatly facilitates this process, yet it is not absolutely necessary.

Having found the number of cells in five great squares, we return to the degree of our dilution and the capacity of the small square as the other factors for the estimation of the number of red cells per cubic millimeter. Suppose our dilution to have been 1 in 200, and the number of red cells found in five great squares (80 small squares), 87, 95, 93, 86, 89 respectively, a total of 450. Factors employed are:

450 = no. of cells.
$\frac{1}{400}$ mm. = area small square.
$\frac{1}{10}$ mm. = depth of small square.
$\frac{1}{200}$ = dilution.
80 = number small squares counted.

Hence 450 × 400 × 10 × 200 = 360,000,000 ÷ 80 = 4,500,000, the number of cells in 1 c.mm. of undiluted blood. A rapid method for arriving at the number of cells per cubic millimeter of undiluted blood (dilution 1–200) is to add four ciphers to the number of cells found in 80 small squares, 450, which gives us 4,500,000.

Leukocyte Count.—The counting of the white cells differs only in that we are dealing with a much lower dilution of the blood, and that we count 400 instead of 80 small squares. The 400 small squares may often be brought into the field under a two-thirds lens, and when the number of leukocytes is not at all great, they may be readily counted. When the number of leukocytes is high, it is necessary to use a one-fifth lens, starting at the upper left-hand corner of the slide, and moving the slide gently so as to move each column of great squares until the entire slide has been covered (Fig. 32). Here again the same precaution of counting all cells touching upon the top and left-hand lines, and of omitting those cells resting upon the right and bottom lines, must be observed.

After counting the cells in 400 small squares, dilution 1–20, see other factors for estimating the red cells. The direct method for estimating the number of leukocytes in 1 c.mm. of undiluted blood is to multiply the number of cells found in the 400 squares by 200. Suppose the number found to be 35, and the dilution 1 in 20: 35 × 200 = 7000. I have not been able to obtain satisfactory results by using the pipet devised for the estimation of the red cells.

Care of the Hemocytometer.—Equally important with other steps in the counting of the blood-cells is the cleaning of the pipet, which should be done immediately after the count is made.

Remove the rubber tube and place it upon the graduated end of the pipet. Force out the contents and fill with distilled water. Rotate rapidly between the thumb and finger, and again expel the water. Repeat this process three or four times; remove the rubber tube and place it upon the end of the pipet to which it belongs; fill the pipet with distilled water, and following the expulsion of this liquid the pipet is dried by blowing through it.

If this method be conducted carefully, there is no need for further cleansing of the instrument. Many prefer to use ether as the last solution placed in the tube, but I have found better results to follow the employment of distilled water. The counting chamber is to be cleansed with water only, and rubbed with a camel's-hair brush.

Caution.—Blood allowed to dry in the pipet is dislodged with great difficulty; and in case the pipet for the white cells becomes darkened, this color is removed by filling the pipet with acid. A particle of blood obstructing the lumen of the pipet may be removed by inserting a bristle into the pipet and then attempting suction. The graduated slide is cleansed in distilled water, care being taken to avoid friction, since the fine rulings are easily destroyed. Blood may be kept in the pipet for twenty-four hours, and if they are thoroughly rotated before expelling any of their contents, the cells will be found equally disseminated throughout the mixture. This avoids the necessity of carrying a microscope to the bedside, and brings hematologic study within the domain of general medicine.

Oliver's Hemocytometer.—With Oliver's hemocytometer (Fig. 33) the estimating of the corpuscles depends upon an optic effect without directly counting them, and for all practical work the actual counting of the cells may be avoided by the use of this instrument, unless there be present a high degree of leukocytosis. The principle of this instrument is based upon the fact that a quantity of blood diluted with Hayem's solution is placed in a test-tube the sides of which are flattened so that the mouth forms a right angle of about 15 by 5 mm. A candle-flame is looked at through the mixture, and when a certain degree of dilution is reached, a bright horizontal line appears on the glass. This line is the result of a number of minute images of the flame produced by the longitudinal striations of the glass, and in case the quantity and quality of the blood used are in every instance the same, the degree of opacity then depends upon the amount of Hayem's solution added. By noting this point one is able to estimate, with a fair degree of accuracy, the number of red corpuscles present in 1 c.mm. of undiluted blood.

Method.—Collect the blood as previously described for estimating hemoglobin. Cleanse the pipet and bring its smaller extremity in contact with the summit of the drop of blood, when it fills by capillary attraction. Wipe away all blood clinging to the outside of the tube. Fill a medicine dropper with Hayem's solution; immediately connect the dropper with the blunt end of the pipet (Fig. 33), and wash the blood into the larger graduated tube. When the hemoglobin has been previously found to be 90 per cent. or more, it is usually justifiable to fill the large tube to the graduation 80; but should the hemoglobin be lower, the dilution should be correspondingly lessened. When the point is neared at which the flame image is likely to appear, the diluting fluid should be added in quantities of five to ten drops at a time, placing the thumb over the mouth of the tube and inverting it several times to effect a perfect mixture of the blood and the additional solution. That portion of the solution which clings to the surface of the thumb should be wiped upon the edge of the tube. When sufficient of Hayem's solution has been added, the image suddenly becomes visible and it is seen more easily by rotating the tube upon its long axis. After the first detection of the image add Hayem's solution drop by

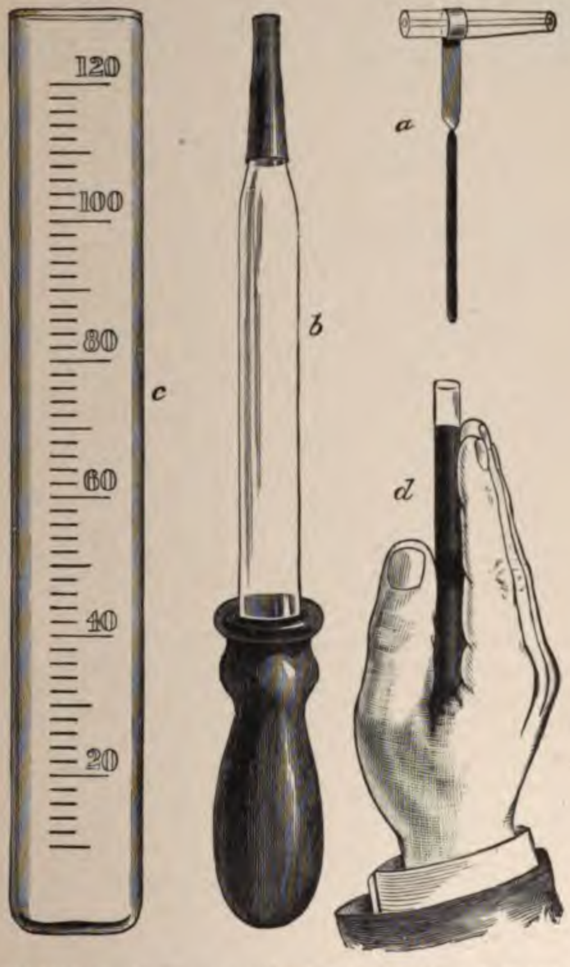

Fig. 33.—Oliver's hemocytometer: *a*, Measuring pipet; *b*, dropper to contain Hayem's fluid; *c*, mixing tube graduated in percentages; *d*, mode of making the observation (this must be done in a dark room); *a*, *b*, and *c* are natural size.

drop until the horizontal line of the flame is visible across the short line of the tube. The appearance of the incomplete line at the sides denotes the approaching reaction. This degree of opacity remains unchanged for several minutes. The whole process needs to be carried out in a dark room, shutting the diffused light of the candle from the eye as shown in figure 33, *d*. The tube should be held near the eye, and the operator stand at a distance of ten feet from the candle. Wax candles are preferable.

ALKALINITY OF THE BLOOD.

The alkalescence of the blood being due to the presence of carbonates, bicarbonates, and albumins held in solution by the acid phosphates, it is difficult to estimate the changes in these several principles, and the variations in the reaction produced by the several processes in alkalimetry. When serum alone is used for the estimation (by titration), the alkaline principles of the clot are not included; and if laked blood is used, uncertain chemic changes are produced which depend upon the delicately balanced albumins and phosphates. It has been clearly shown that there exists in the blood—and in the serum—certain rather constant alkaline principles which are sufficiently closely estimated by the processes about to be described as to render this knowledge of clinical value. Again, it is evident that the degree of difference between the alkalinity of normal and that of diseased blood displays rather wide variations. During life the reaction of the blood is alkaline owing to the presence of disodium phosphate and sodium carbonate. The degree of alkalinity under normal conditions is estimated in terms of sodium hydrate, and corresponds to 182 to 218 mgm. for every 100 c.c. of blood. Von Jaksch, however, makes a higher estimate of alkalinity—260 to 230 mgm.; while Canard places it at 203 to 276.

Clinical Significance.—*Decrease.*—The alkalinity of the blood is low in women, children, and during the latter stage of digestion (when the hydrochloric acid and peptones are reabsorbed) and after violent exercise. Pathologically, a reduction occurs in severe anemias (primary or secondary), leukemia, pernicious anemia, chronic hepatic disease, nephritis, diabetes, pseudoleukemia, and in the cachexia resulting from cancer. High fever, general toxemia, the prolonged use of acids, and poisoning by acids or carbon monoxid are attended by a low alkalinity, as are also acute mania (stage of excitement) and epilepsy. In the latter it begins just prior to a seizure, and continues to fall after the convulsion, the reduction varying according to the muscular contractions during the convulsion.[*] The normal alkalinity is restored five to six hours after a convulsion.

Increase.—An increased alkalinity is to be found early during the process of digestion and after a cold bath.

Kireew[†] in studying the blood of 25 cases of typhus fever found the alkalinity to increase with the advance of the disease, and to reach its maximum with the height of the disease. The alkalinity fell rapidly to normal in favorable cases In three fatal cases the alkalinity remained above normal.

Estimation of Alkalinity.—Lowy's Method.—1. Place 45 c.c. of a 0.25 per cent. solution of ammonium oxalate in a flask having a graduated neck.

2. Collect 5 c.c. of venous blood from the arm into a syringe (see *Bacteriology of Blood*) and immediately transfer it to the sodium solution, which prevents coagulation and dissolves the hemoglobin from the protoplasm of the erythrocytes. Titrate the mixture with a 0.04 per cent. solution of tartaric acid. Lacmoid paper that has been previously soaked in a concentrated solution of magnesium sulphate serves as an indicator. The $\frac{1}{25}$ normal solution contains 3 gm. to the liter, and 1 c.c. of this solution therefore corresponds to 0.0016 gm. of sodium hydrate; *e. g.*, 10 c.c. of the $\frac{1}{25}$ normal solution were required to neutralize 5 c.c. of blood—the alkalinity of this quantity of blood in terms of sodium hydrate is 0.016 gm. To determine the alkalinity of 100 c.c. of blood, multiply 0.016 by 20 (the portion of 100 c.c., $\frac{1}{20}$ being employed) which equals 0.32 gm.

Reagents.—Normal solution of tartaric acid (75 gm. to the liter).

Lacmoid paper is prepared in the following manner: The mixture is made of 100 gm. of resorcin, 5 gm. of sodium nitrate, and 5 c.c. of distilled water, which when heated on an oil bath to a temperature of 110° C. gives a violet color. The flame is removed before this temperature is reached. Again heat to 115°-120° C. until the ammonium ceases to be evolved. Dissolve the pure blue residue in water and precipitate with hydrochloric acid, cool, filter with the aid of a suction pump, and wash with a small quantity of water. Again dissolve in absolute alcohol, filter, and evaporate in an ammonia-free atmosphere. This pigment crystallizes in reddish-brown glistening plates; one gm. of which is dissolved in 1000 c.c. of 45 per cent. alcohol. Immerse narrow strips of Swedish filter-paper in this solution for several minutes, dry them in the air, and place in a well-stoppered bottle. As this article is so seldom required, and as it necessitates such labor, it might be more advantageous for the student or practitioner to have this paper prepared by a chemist.

Dare's Hemoalkalimeter.—Dare's instrument (Fig. 34) consists of a glass tube (A) provided with a glass stopper (B), and through it passes an automatic capillary pipet which tapers at its exposed point. This capillary pipet has a capacity of 20 c.mm. (15 mgm. by weight) of blood. The stopper and the capillary pipet are fitted tightly into the tube (A), which is graduated to 3 c.c. to represent the equivalents in milligrams of NaOH to 100 c.c. of blood. The upper end of the tube is expanded, and upon this expansion is a minute opening (C) for the admission of air. A medicine dropper graduated to 2 c.c. and provided with a piece of rubber tubing which is applied to the exposed end of the capillary tube serves to introduce the test solution. The spectroscope is necessary, and Browning's pocket instrument will be found satisfactory.

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[*] Pugh, "Jour. Mental Science," Jan., 1903, vol. xliv, p. 71.

[†] "Medizinskoe Obosreni," lxii, No. 19.

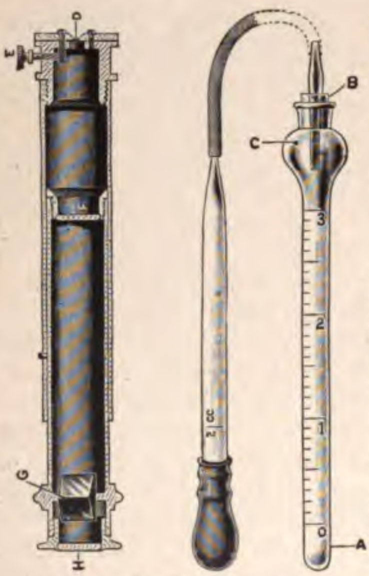

Fig. 34.—Dare's hemoalkalimeter.

Test solution:

Tartaric acid (Merck's reagent)	0.075	gm.
Alcohol (94 per cent.)	20	c.c.
Distilled water . . . . q. s.	200	"

Method of Application.—(1) Obtain a drop of blood from the tip of the finger; hold the tube horizontally and permit the exposed tip of the capillary pipet to touch the summit of a rather large drop of blood, when it fills by capillary attraction. (2) Hold the tube vertically and wash the contents of the pipet into the tube by forcing the distilled water from a medicine dropper through the pipet. This washing is continued until the distilled water, collected at the bottom of the tube, reaches the graduation zero (0). (3) Close the opening of the tube by the finger and invert the tube repeatedly until the blood and distilled water are thoroughly mixed. (4) A dropper is now filled with the acid reagent, and this solution is forced through the capillary pipet into the tube.

Caution.—Cover the opening in the expanded portion of the large tube before releasing the pressure from the rubber bulb of the dropper. (5) Without detaching the dropper, hold it in a vertical position and invert the tube several times. (6) Place that portion of tube (A) below the graduation (0) in the cleft of the spectroscope, and examine for the existence of the absorption-bands of oxyhemoglobin (see *Spectra*, Fig. 24, page 44). The acid solution should be added carefully after each examination until these bands disappear. The bands will be noticed to fade gradually as the point of neutralization is approached. Invert the tube (A) after each additional drop of the acid solution.

Upon the disappearance of the bands of oxyhemoglobin the test is completed. Note the result from the scale on the tube (A), which is graduated in both cubic centimeters and the equivalents expressed in milligrams of sodium hydrate to 100 c.c. of blood.

"SCALE OF EQUIVALENTS COMPUTED FROM A BASIS OF 15 MGM. OF BLOOD TO 2 C.C. OF ACID SOLUTION. ONE TWO-HUNDREDTHS OF THE NORMAL.

Cubic Centimeters of Reagent.	Milligrams of NaOH to 100 Cubic Centimeters of Blood.
2.6	345.0
2.4	319.0
2.2	292.0
2.0	266.0
1.8	239.0
1.6	212.0
1.4	176.0
1.2	169.0
1.0	133.0
0.8	96.0
0.6	79.0
0.4	53.0
0.2	26.6

"The equivalent weight of tartaric acid $\frac{H_2C_2H_4O_6}{2} = 149.64$. Tartaric acid being dibasic, one-half the number of molecules are taken to make a normal solution which equals 75 gm. (approximately) to a liter; for the present solution there is taken $\frac{1}{200}$ of the normal, which is equivalent to 375 mgm. to a liter. Sodium hydrate has 40 atoms to its molecule, which gives an equivalent of 40 gm. to a liter; therefore, $\frac{1}{200}$ of the normal tartaric acid solution will give an equivalent of 0.0002 mgm. of the alkali. Every 100 c.c. of blood will equal 266 mgm. of sodium hydrate" (Dare).

For every 2.2 c.c. of reagent employed the equivalent of 292 mgm. of sodium hydrate to 100 c.c. of blood is read from the graduation on tube (A). Dare employed open gaslight in his observations.

ACIDITY OF THE BLOOD.

Certain of the salts in the blood are not saturated; *e. g.*, $NaHCO_3$ and NaH_2PO_4. It is noteworthy that these give an alkaline reaction to litmus, but an acid reaction to phenolphthalein, and are capable of combining with bases. Fresh blood is alkaline, while serum causes an acid reaction with phenolphthalein. The *basic capacity* of the blood is the power its salts possess to neutralize bases. Kraus has estimated the basic capacity of normal venous blood at 0.162 to 0.232 per cent. NaOH.*

Clinical Significance.—An increase has been observed in fevers of from 0.209 to 0.272 per cent. NaOH ; and to reach 0.347 per cent. in diabetes (Kraus).

COMPOSITION OF THE WHOLE BLOOD.

Albumins.—The albuminous principles in the normal blood as found in the circulation are hemoglobin, serum-albumin, serum-globulin, and fibrinogen. Shed blood contains a nucleoproteid principle derived from the nuclei of leukocytes or from the red cells; and this substance combines with the calcium salts to form fibrin ferment ("prothrombi"). In certain diseased conditions (*e. g.*, brain abscess) a small quantity of albumose has been found. The entire proteid group of the blood has been estimated by von Jaksch at 22 per cent.; Limbeck rates it at about 25 per cent., and Schmidt records it from 10.82 to 16.63 per cent.

Clinical Significance.—A relative increase of albumin is occasioned by marked loss of fluids; *e. g.*, in cholera and diarrhea; also in acute articular rheumatism, pleurisy, pneumonia, erysipelas, and other acute inflammatory processes.

Reduction of the total albumin may accompany infectious diseases with high fever, even though the number of red cells remains normal; and early in chronic nephritis, in pernicious anemia, malaria, leukemia, pyemia, and in secondary anemia.

Peptone.—Peptone, a constituent of cadaveric blood, does not appear to have been satisfactorily demonstrated in the circulating blood. However, when blood is allowed to stand for several hours, a reaction for peptones may be obtained. Albumose has been found in leukemic blood when the eosinophilic cells were numerous. We may here allude to the great similarity of the reactions caused by the albumose and those produced by globin (see *Albumosuria*, page 216). Quantitative estimation of the total albumin of the blood has not been considered, and the reader is referred to special works on Chemical Physiology and Pathology for specific knowledge upon this subject.

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* "Zeit. f. Heilk.," vol. x, p. 106; also "Arch. f. exper. Path.," vol. xxvi.

Inorganic Substances of the Blood.—Here again the subject falls outside the scope of this chapter. The estimation of the quantity of the blood ash has been briefly described, but for other and complete description the reader is referred to special works on Physiology and Pathology.

Urea.—Traces of urea are normally to be found in the blood, 0.016 to 0.02 per cent. (Simon), and an increase is observed in fevers, cholera asiatica, cholera infantum, eclampsia, and also in renal conditions when it is imperfectly eliminated by the kidneys. For determination and the detection of urea in the blood see *Urine* (Hoppe-Seyler's method).

Uric Acid.—Traces of uric acid are demonstrable in normal blood. An increase has been observed in appreciable amounts, varying greatly with the type of disease present, during the course of pneumonia, anemia, cardiac embarrassment, acute gout, leukemia, nephritis (88.88 per cent. of cases, v. Jaksch). It has been shown that an increased elimination of the uric acid through the urine is accomplished by an increased quantity of uric acid in the blood, but this condition is not constant. Lowered alkalinity of the blood in the infectious fevers stands as an exception to this rule.

Method of Detection.—Obtain 100 c.c. of blood (see page 34) and immediately transfer to a beaker. Dilute with three or four times its own volume of distilled water, and heat on a water-bath. When coagulation begins, a 0.5 per cent. solution of acetic acid is added in small quantities until a faint acid reaction is given. Continue the heat for twenty minutes, when the albumin will be found precipitated as brownish particles. Filter hot, heat, and wash the precipitate with hot water. Again add small quantities of acetic acid solution and bring both filtrate and washing to the boiling-point. Decant, filter, add a small quantity of disodic phosphate, and continue according to Ludwig-Salkowski's method (see page 198). Treat the first filtrate with hydrochloric acid and evaporate to about 10 c.c. Allow to stand for twenty-four hours and separate the precipitated uric acid by filtering, and continue examination of the filtrate for uric acid (see *Urine*). The murexid test (see page 196) may be applied directly to the fluid before the uric acid has had time to precipitate; and its employment is necessary when no uric acid separates from the solution after standing for twenty-four hours.

Glycogen.—Blood smeared upon slides or cover-glasses in the usual manner and dried in the air is stained for from three to five minutes with the following: Iodin, pure, 1 part, potassium iodid 3 parts, water 100 parts, pulverized acacia in excess. In the presence of glycogen there are a number of small granules of a mahogany-brown tint in the leukocytes and occasionally in the plasma. Question has arisen as to whether or not these granules represent subjects more closely allied to amyloid than to glycogen. The extracellular granules are both fine and coarse, varying from $\frac{1}{6}$ μ in diameter, and appear to be the only form found in the normal blood. An increase in the number of granules as seen in disease is shown by intracellular granules. Extracellular granules are possibly derived through degeneration of leukocytes. Neutrophilic leukocytes have been known to contain these granules in case of leukemia and diabetes, and they have been found in the plasma in connection with pathologic conditions. After a review of the current literature on this subject it is evident that further observations are needed to establish any valuable clinical data.

Fat (Lipemia) and Fatty Acids.—Normal blood contains between 0.75 and 0.85 per cent. of fats.* Particles of fat may be demonstrated in the blood by fixing the films in a one per cent. solution of osmic acid for twenty-four hours, and staining from one-half to one minute with a one-half of one per cent. aqueous solution of eosin. The particles of fat having stained black with the osmic acid, the remainder of the field takes the eosin stain (Fig. 35). In view of the fact that all granules staining black may not be fat a control preparation is necessary, and should be conducted as follows: Fix the film twenty-four hours in alcohol and ether, and then in the osmic acid for twenty-four hours; counterstain with eosin, extract the fat by ether, and the absence of black particles in the cells and plasma is evidence that the blackening displayed by the former specimen was due to fat. Free fat (palmitin, stearin, and olein) may be detected in the blood in health and in disease, but it is usually present in comparatively small amounts, and while recognized with some difficulty under an oil-immersion objective, the granules are at times conspicuous.

Quantitative Estimation.—Collect from 5 to 10 gm. of blood and dilute with 100 to 200 gm. of 96 per cent. alcohol; mix thoroughly, allow to stand for twenty-four to forty-eight hours, and filter. Subject the deposit collected on the filter to a repetition of the above treatment. The part remaining is treated with ether, and the remainder digested and thoroughly shaken with ether. Collect the various alcoholic and ethereal extracts, evaporate slowly to dryness, extract with absolute ether, dry, and weigh (Hoppe-Seyler). Extract filter-paper to recover any fat it may have withheld (see special works on physiologic chemistry).

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* Bonninger, "Zeit. f. klin. Med.," 1000, vol. xli, pts. 1 and 2.

Clinical Significance.—The quantity of fat in the blood is increased after a heavy meal and in acute alcoholism. An excess of 0.05 to 0.16 per cent. has been found in the blood of diabetes, 0.1 to 0.5 per cent. in nephritis, 0.15 per cent. in pneumonia, and 0.16 per cent. in typhoid fever. An increased quantity has been observed in starvation, phthisis, fatty embolism, carcinoma of esophagus, and poisoning by carbonic oxid. A fat-splitting ferment has been detected in the blood.[*] V. Jaksch has demonstrated fatty acids in the blood of diabetic coma, acute yellow atrophy (hepatic), acute infections, and leukemia.

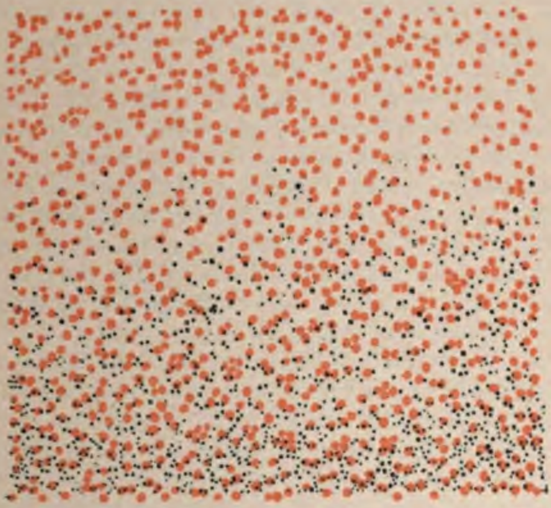

Fig. 35.—Blood from a case of lipemia, stained with osmic acid: upper half of field cleared with oil of turpentine; lower half shows the fat-droplets and granules stained with osmic acid between the blood-corpuscles; enlargement, 100 diameters (Gumprecht).

Glucose.—Normal blood contains a trace of glucose, and this quantity may fluctuate, depending upon a diet rich in carbohydrates (increase) or by muscular exercise and hunger (decrease). It has been found in the blood of health by Limbeck five hours after a meal (0.075 to 0.089 per cent.). Freund and Trinkler found glucose increased in the blood from cases of cancer, and were able to reduce cupric oxid when exempt from albumins. Trinkler found 3 per cent. of glucose in the blood of carcinoma.

[*] Hanroit, "Compt. Rend. Soc. Biol.," 1896, p. 925.

Depending upon the character and stage of the disease, glucose has been found to reach 0.9 per cent. in the blood of diabetes.*

Estimation of Glucose in the Blood.—A quantity of blood is weighed, freed from albumin by boiling with an equal quantity of sodium sulphate, and then filtered; the precipitate is washed carefully, and the filtrate treated with Fehling's solution. A more positive demonstration is by polarimetry (see page 236). The blood should be fresh, lest the glycolytic ferment produce misleading reactions. It is not known upon what the glycolytic principle of normal blood depends.

Diabetic Blood.—A test for diabetic blood worthy of description is Williamson's. Knowledge obtained from any special reaction of the blood is impracticable unless it enables us to recognize diabetes at an earlier period than can be done by urinalysis; and the majority of writers on this subject hold that this has not been proved. Diabetic blood is rich in fats.

Williamson's test is easy of execution, and consists in the decolorizing of an aqueous solution of methylene-blue. Collect two drops (20 c.mm.) of blood, and dissolve in 40 c.mm. of water; then add 1 c.c. of methylene-blue solution (1–6000) and 40 c.mm. of liquor potassæ (specific gravity 1.058). Place the vessel containing the mixture in boiling water for four minutes. The solution is decolorized with diabetic blood, and remains a deep blue with normal blood.

Clinical Significance.—Williamson has been unable to obtain this reaction with the blood from other forms of disease. The reaction is produced by diabetic urine. Williamson obtained positive reaction in nine cases of diabetes, and negative results in 130 non-diabetic cases. Adler confirmed the validity of Williamson's reaction, and attributes it to reduced alkalinity of the blood. He has also shown that Bremer's test for diabetes is not of diagnostic value.

Red Cells.—Sugar in the blood extracts water from the tissues into the vessels, thus diluting the blood; but blood concentration is soon accomplished owing to the increased diuresis. It is the influence of these processes that is responsible for the wide variation in the number of cells found per cubic millimeter, and on this account it is impossible to arrive at any constant number of erythrocytes for this disease. Even during the period of cachexia and anemia in diabetes blood concentrations may be found well marked, and the number of red cells high. The hemoglobin may fluctuate between wide limitations and is doubtless dependent upon similar conditions.

* Hoppe-Seyler, "Physiological Chemistry," 1881.

Leukocytes.—The number of leukocytes varies greatly in different cases and at different times during the course of the same case; yet leukocytosis has not in my experience been a common occurrence, though the leukocytes have been seen to reach 10,000 to 12,000 per cubic millimeter. Cabot did not observe leukocytosis in the study of thirteen cases, and during diabetic coma he found the leukocytes to number 4200 per cubic millimeter, while in another, a child of eight years, there were 49,000 leukocytes present.

Acetonemia.—It has been found by Deichmüller and by v. Jaksch that by extracting the blood with ether and by distillation a principle could be isolated which gives a reaction for acetone. This principle was found to be increased in fevers.

Cholemia.—This term has been applied to the presence of biliary acids in the blood, yet such bloods commonly contain cholesterin.

Icteric blood appears to the naked eye as yellowish in color, and in its serum or foam may be seen small particles of bile-pigment. The application of heat (70° to 80° C.) causes the red bilirubin to be replaced by a greenish tinge—biliverdin. Limbeck's analyses of icteric blood show an increase of nitrogens from 3.29 to 3.52 per cent., while the chlorids were diminished both in the blood and in the serum. An increase in the volume of red cells is mentioned.

Isotonic Tension.—Low isotonic tension and increased resistance of the red cells are peculiar to jaundice (see page 74). The effect exercised by the bile acids upon the hemoglobin probably renders it more freely soluble. Bilirubin may be demonstrated in the blood when urobilin exists in the urine.

Method.—Collect the blood either by a hypodermic syringe from the vein or by a wet cup, place in a bowl, allow to coagulate, and after two or more hours decant serum and filter. The filtrate displays a yellow tint when bilirubin is present, and becomes green after heating for several hours at 35° C. A mere trace of bile-pigment is detected in this manner.

Biliary Acids in the Serum.—Pettenkofer's Method.—Remove the albumins by boiling or by alcohol; filter and treat the filtrate with lead acetate and with ammonia. The acids are precipitated with lead compounds. Wash the precipitate on a filter, boil in alcohol, filter, and decompose the lead salt by carbonate of soda. Again filter, and evaporate to dryness. The acids are extracted by boiling in absolute alcohol. Evaporate the alcoholic extract when biliary acids crystallize, and an amorphous substance remains from which crystals are obtained after extracting with ether (see works on physiological chemistry).

Globulicidal Properties of Serum.—Serum of pathologic blood when brought in contact with normal blood often causes the red cells to dissolve and the hemoglobin to be replaced by a greenish hue. Spectroscopic examination reveals the presence of hematoidin instead of hemoglobin in such mixtures. Globulicidal properties may be well marked in the serum of the primary anemias, purpura, malaria, leukemia, erysipelas, typhoid fever, pneumonia, and nephritis. Buchner has shown that the globulicidal activity is more marked in serum poor in salts, and that it is also diminished after the addition of salts. A close relation must exist between the globulicidal property of the serum. The blood of one animal brought in contact with the blood of another animal displays coagulative power, which may be greatly lessened by the addition of salt or by heating to 50° C.; yet these do not totally destroy any toxicity that may exist in the serum. Toxicity of the serum depends principally upon certain retained albuminoid bodies. Wide variations in the toxicity, globulicidal and coagulative properties of different serums may be found in both health and disease (see *Serum-diagnosis*, page 116).

Diastatic Ferment of Blood.—The power of the blood in both health and in disease to digest starch has recently received clinical attention. To demonstrate this property add 1 c.c. of blood to 50 c.c. of starch solution, stand several hours in an incubator, and test for glucose with Fehling's solution. Two c.c. of normal human blood when added to 50 c.c. of starch solution and kept at a temperature of 30° C. for twenty-four hours was found by Castellino and Pracca to yield 0.07 per cent. of sugar. Diastatic fermentation is most active in arterial blood that is kept at a temperature of 30° to 38° C., and inhibition is observed at 75° C. (Cavazzani).

Clinical Significance.—The fermentative power of the blood is increased in chlorosis, leukemia, nephritis, pneumonia, malaria, hepatic cirrhosis, and carcinoma, and was found decreased in other forms of disease.* The addition of nuclein inhibits the diastatic ferment, while sodium sulphate and sodium chlorid intensify it.

CRYOSCOPY OF THE BLOOD.

Cryoscopy as applied to the body fluids and secretions, especially to the blood, has, as yet, yielded but few definite results. Waldvogel's description† of the technic for determining the freezing-point of fluids is in brief as follows: Pour 8 to 10 c.c.

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* Cavazzani and Pracca, "Arch. p. l. sci. med.," vol. xvii, No. 6.

† "Arch. f. exper. Path.," vol. xlvi, 1 and 2.

of the blood or urine into a small test-tube, around the top of which a rubber ring has been placed. This tube fits into a larger one with a space of about one centimeter between the bottoms of the tubes, and of 2 or 3 mm. between the walls. Both are then placed together in the freezing (ice and salt) mixture (Fig. 36). The temperature is determined by a small thermometer, graduated in fiftieths or hundredths of a degree C. to four degrees below

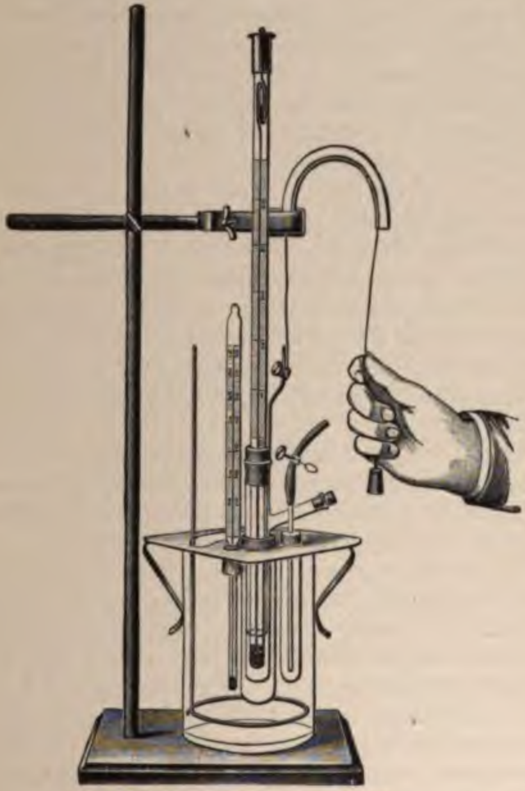

Fig. 36.—Cryoscopic apparatus.

the freezing-point, which is inserted in the blood or the urine. The thermometer is surrounded by a rubber ring which prevents it touching either the bottom or the wall of the tube. When it registers —0.52° C., the fluid is stirred with the thermometer, and it congeals almost instantly into a solid mass of ice.

Diluting the fluid a little does not interfere with the determination of the freezing-point. Blood must be placed on ice or in a refrigerator for forty-eight hours before it is tested. The zero must correspond to the freezing-point of distilled water as verified from time to time. For method of obtaining blood for cryoscopic study, see *Bacteriology of Blood.*

The molecular concentration of any liquid is expressed in its freezing-point, which lowers proportionately to the degree of concentration. Osmosis, too, has an important bearing on this phenomenon, since the osmotic pressure between fluids separated by a membrane is directly proportional to the molecules held in suspension; thus the freezing-point of a solution containing several substances is lowered to the equivalent of the sums of the freezing-points of each substance suspended in the liquid. Human blood under normal physiologic conditions displays a fixed freezing-point —0.56° C.—below distilled water (Kummel).

Clinical Significance.—When the metabolic products are retained in the blood as a result of renal insufficiency, its molecular concentration is increased and consequently its freezing-point lowered, as has been found in the blood of nephritis, hydronephrosis, pyonephrosis; and experimentally in animals after ligation of the ureters. The freezing-point of the blood is unchanged when the remaining kidney compensates after the removal of its fellow.

When the freezing-point of the blood is lowered to — 0.58° to —0.61° C., both kidneys are diseased, and surgical operation of any kind should be interdicted until the freezing-point is about — 0.56° C. A freezing-point of the urine less than — 0.9° C. indicates kidney insufficiency, and whenever one kidney is supposed to be affected, the freezing-point of the urine obtained by catheterization of the ureters or by segregation may be of clinical value. Urine from the diseased kidney will congeal at a higher point than will that from its fellow; *e. g.*, urine from the diseased kidney freezes at — 0.50° C., and urine from a healthy kidney at — 1.75° C. Generally speaking, it is impossible to draw definite deductions from the estimation of the freezing-point of the urine, owing to the wide physiologic limits between which it may fluctuate—from — 0.1° to — 2.0° C. under normal conditions.* Tinker has recently averred the value of cryoscopy as an index to renal insufficiency.† The method of Claude and Balthazard has recently appeared in a detailed translation by F. Burthe.‡ Thus far the methods of cryoscopy are given for physiologists' use only, and are not readily adapted to clinical use.

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* Kummel, "Verhandlungen der deutsch. Gesellsch. f. Chir.," 29th Congress.

† "Johns Hopkins Hosp. Bul.," June, 1903, p. 162.

‡ "Med. News," Jan. 23, 1904, p. 149.

OSMOTIC PROPERTIES OF THE BLOOD.

The red cells will be found to dissolve promptly when a drop of blood is placed in distilled water, but placed in a solution of salt, of sufficient concentration, the red cells retain their hemoglobin, and are precipitated at the bottom of the liquid. This solution of the red cells is the result of the law of osmosis: *i. e.*, when two solutions of different concentrations are separated by an animal membrane, these solutions pass through the membrane until they are of equal densities, or the quantity of salt in each is equal. The force producing this interchange is termed "osmotic tension"; and the two liquids containing an equal quantity of salt are termed "isotonic." Fluids containing a greater or lesser quantity of diffusible salts than other solutions are termed "hyperisotonic" or "hypisotonic," and possess the faculty of extracting water from or yielding it to such fluids in accordance with the laws of osmosis. The red corpuscles in a normal specimen of human blood are prevented from solution when added to a 0.46 per cent. solution of NaCl. We therefore regard the isotonic tension of human red corpuscles to be 0.46 per cent. NaCl. In this solution the red cells swell, absorb water, but do not give up their hemoglobin; and when placed in sufficiently strong solutions, the cells shrink, yielding water to the fluid.

Normal Salt Solution.—A 0.9 per cent. solution of salt prevents either swelling or shrinking of the red cells. Therefore this strength solution represents the isotonic tension of the blood plasma, and is universally known as the *normal salt solution.*

Lowering of the osmotic tension of the plasma of necessity leads first to swelling and eventually to solution of the red cells. The hyperisotonic property of the plasma is a physiologic safeguard for the preservation of the red cells after the introduction of large quantities of water into the circulation (hypodermoclysis). There may occur variations in osmotic tension which affect the volume of the red cells. Certain other diffusible principles, as albumins, also influence the isotonic relation of the blood; which shows that its isotonic properties do not depend entirely upon the presence of salt. Limbeck found only 0.2 per cent. of salt in the red cells, while their isotonic tension equaled at least 0.46 per cent. NaCl. It has been further shown by Hamburger * that albumins, chlorids, and phosphates appear differently when under varying osmotic conditions.

The addition of acid to blood causes phosphates and albumins to pass from the erythrocytes into the serum and the chlorids change from the serum into the cells: the reverse phenomena follow the addition of an alkali. Probably most important among the functions of osmotic tension is the influence it exercises in causing the hemoglobin to be contained in the red cells.

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* "Arch. f. exper. Path.," vol. xii, p. 237.

Clinical Significance.—Rather wide physiologic variations in the isotonic tension of the blood are to be observed normally; and that of the venous blood is regarded as being slightly higher than is that of arterial blood. It is increased by the addition of hydrogen, nitrogen, arsenic, CO, CO_2, and acids; and is diminished by a trace of alkalis or oxygen. During the course of typhoid fever, pneumonia, erysipelas, and other acute infections, the isotonic tension may be increased (Limbeck). It was also found increased in leukemia, secondary anemia, pregnancy, and lactation; but low in jaundice and in chlorosis.

STUDY OF FIXED AND STAINED BLOOD.

SLIDES AND COVER-GLASSES.

The first step in the preparation of smears or films upon either cover-glasses or slides has been previously described on page 35, and such spreads are fixed in one of the following manners: The method I have found most satisfactory and available is the use of a strip of copper about ¼ x 4 x 28 inches (Fig. 37), which is allowed to balance on a tripod, with the flame of a Bunsen burner beneath one end of the copper. After the copper has been thoroughly heated, fill a pipet with cold water and drop it on the copper at different distances from the flame. At the point where the water boils, but is not excited into bubbles that bounce off the stage, place the cover-glasses or slides, specimen down, and allow them to heat for twenty minutes. They are then removed, labeled, and placed in small pasteboard boxes, where they may be kept for an indefinite time.

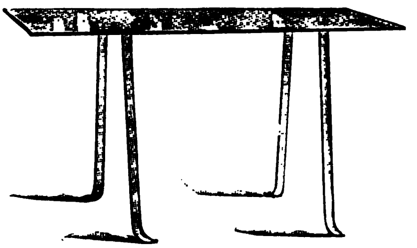

Fig. 37.—Stage for fixing blood.

Spreads may also be fixed by placing them in a Petri dish, specimen up, and this dish set within a larger dish containing a small quantity of 40 per cent. formalin. Cover the larger dish and permit the surface of the smears to be exposed to the fumes of the formalin for from twenty minutes to a few hours. Results from this method of fixation are, at times, unsatisfactory.

Grasp a cover-glass or a slide containing a spread in the forceps and pass it rapidly through the flame of a Bunsen burner, care being taken that after each insertion into the flame the surface of the glass is touched upon the palmar surface of the hand. A guide to the degree of heat applied is obtained in this way, and after four or five applications to the flame the specimen is often well fixed; yet considerable skill is necessary for the successful employment of this method.

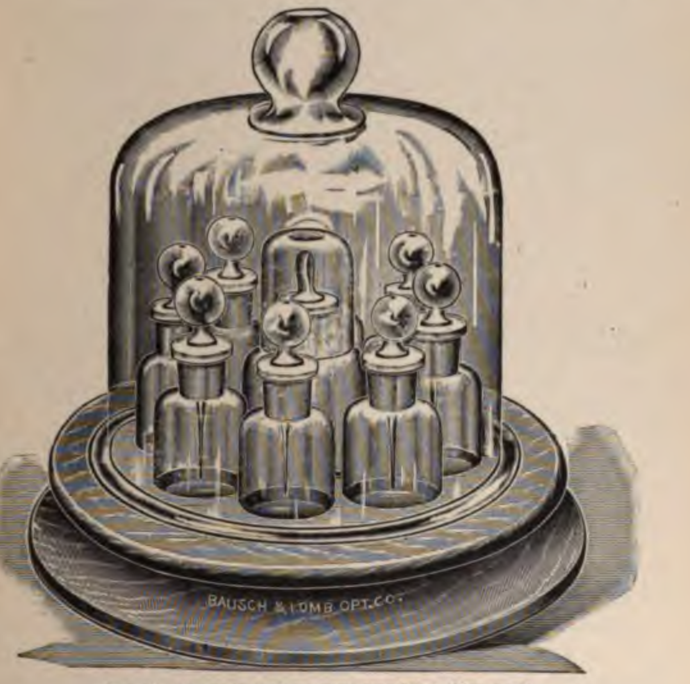
BAUSCH & LOMB OPT. CO.

Fig. 38.—A convenient reagent stand for office work.

Arrange the smears in a Petri dish, cover, and allow to stand in an incubator, at a temperature of 37° C., for twenty-four hours. A fixing solution containing—

Osmic acid	1	gm.
Sodium chlorid	0.6	"
Distilled water	100	c.c.

may be applied at the time the films are made. (1) Wash a small camel's-hair brush in alcohol to remove all fats. (2) Immerse the brush in the fixing solution, and then touch it to the summit of the drop of blood, when the blood is disseminated through the brush, and is acted upon by the osmic-acid solution. (3) Smears are made by drawing the tip of the brush over the surface of a slide or cover-glass and are permitted to dry in the air.* A number of films may be made after a single application of the brush to the blood.

STAINING.

The most essential points in the staining of blood are the results obtained by a given method and the simplicity of the method employed. In 1891 Romanowsky detailed a method for the staining of malarial parasites by which the chromatin and the cytoplasm were stained differently. Since the appearance of Romanowsky's original paper many have tried to perfect a similar staining method. Jenner detailed a most practical stain for blood; and Leishman simplified to some extent Jenner's method. It was not until 1902 that J. H. Wright gave to the profession a practical application of this method.

Eosin and Hematoxylin.—It has been my custom to employ this stain almost entirely in class work and rather extensively in my general clinical studies. The solutions necessary for this method of staining the blood are: first, one-half of one per cent. of eosin in 70 per cent. alcohol; and, second, Delafield's hematoxylin:

Hematoxylin crystals	4 gm.
Alcohol (absolute)	25 c.c.
Ammonia-alum crystals	52 gm.
Distilled water	400 c.c.
Glycerin	100 "
Methyl-alcohol	100 "

Preparation.—Place the alcohol in a mortar, add the hematoxylin crystals, and rub until well dissolved; then place in a clear glass bottle, cork loosely, and allow to stand exposed to the light for four days. Add the ammonia-alum crystals to the water and treat in a similar manner. At the end of four days mix these two solutions, shaking thoroughly, and after a few hours filter. The glycerin and methyl-alcohol are now added, and the mixture allowed to stand for several hours. The solution is now filtered, placed in a clear bottle, and allowed to stand exposed to the sunlight for six weeks (ripening process), when it will be found ready for use.

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* Kornilouitch, "Vratch," Nov. 17, 1901, vol. xxii, No. 46.

Application of Stain.—First stain the specimen with the eosin for one-half minute; wash in water. Then, without drying, stain with hematoxylin for from one to three minutes, as the time varies greatly with different stains even though they be prepared in essentially the same manner. Wash and dry the specimen, and mount as before described.

Instead of hematoxylin, a two per cent. aqueous solution of methylene-blue may be employed for one-half minute. A mixture of eosin and hematoxylin has been suggested, but in my hands it gave unsatisfactory results; a mixture of eosin and methylene-blue is serviceable for staining the malarial parasite. The eosin and hematoxylin method possesses certain properties necessary for the study of the finer structure of the nuclei, karyokinetic figures, and basophilic granules, but is not generally conceded to be as valuable as Ehrlich's mixture for diagnostic purposes. In the greater part of clinical work, however, the former method will be found to be most reliable and satisfactory.

MICROSCOPIC EXAMINATION OF THE STAINED BLOOD.

It is possible to determine the character of the specimen by a two-thirds or one-sixth objective, but in order to bring out clearly and to study in detail the morphology of the finer structures of the cells and their granules a one-twelfth oil-immersion objective is required.

Normal Blood.—Normal human blood when stained with eosin and hematoxylin displays certain characteristics which distinguish it quite clearly from the blood of animals (Plate 2), but disease is capable of producing retrograde changes, in which case the red cells, principally, are distorted and resemble those found in the lower forms of animal life; such changes depending entirely upon the variety and degree of disease present. Physiologically, this condition is observed in blood from the umbilical cord of the fetus—an advantageous source from which to prepare spreads for class work, since nucleated red cells are present in considerable numbers (Plate 2).

Fixing and Staining Combined.—Jenner's method for these two steps in hematologic technic deserves more than passing recognition. In the author's hands it has proved entirely satisfactory, and it has been further found that good results attend the use of this stain for specimens previously fixed by heat. Jenner's solution is applied in the usual manner to an unfixed film, and permitted to stain for from one to three minutes; wash in water, ten to thirty seconds, until a pink tint appears. The red and white cells, nuclei, and granules are brought out clearly and definitely (Plate 7). The malarial parasite stains deeply (red), as a rule, but Jenner's method* cannot be said to equal that of eosin and hematoxylin or of Nocht's method.† F. C. Wood‡ has reviewed the literature in reference to the various modifications of Jenner's method, and in addition suggests a rapid stain for the malarial parasite, the preparation of which stain can be found by reference to Wood's article.

Jenner's Stain.—Place equal parts of an aqueous solution of yellow eosin (1.25 per cent.) and of an aqueous solution of medical methylene-blue (1 per cent.) in a rather broad dish, mix thoroughly, cover, and allow to stand for twenty-four hours. Filter, and dry in an oven at 55° C. or in the air. The residue clings to the filter-paper, which is now to be pulverized and washed in distilled water. Shake well, and again wash upon a filter; dry, powder, and place in a bottle. Dissolve 0.5 gm. of powder in 100 c.c. of methyl-alcohol (Merck's alcohol for analytic use). G. Grubler's stains should be used.

Wright's Method.—The blood when smeared on slides or cover-glasses is allowed to dry in the air. The spread blood does not stain well after it is exposed to the air for several months.

1. Add to the specimen sufficient of an alcoholic solution of the stain to cover the film, and allow it to stand for one minute, to fix the corpuscles.
2. To the alcoholic solution of the stain now on the specimen add water, drop by drop, until the stain becomes semitranslucent and a yellowish, metallic scum forms on the surface. Permit this diluted stain to cover the specimen for two or three minutes.
3. Wash the heavily stained specimen in water until the film of blood gives a yellowish or pink tint to the naked eye.
4. When the desired tint is reached, dry immediately between blotting-paper, lest decoloring be carried too far. The specimen is now ready to be mounted in Canada balsam.

Microscopic Appearance of Stained Blood.—The red cells are orange or pink, and their nuclei are deep blue and their cytoplasm presents a bluish tint. Granular basic degeneration of the red cells is made evident by this stain. Polynuclear neutrophilic leukocytes show dark-blue or dark-lilac colored nuclei and the granules are of a reddish-lilac color.

Lymphocytes have dark, purplish-blue nuclei; the cytoplasm is robin's-egg blue, and in it are seen a few dark-blue or purplish granules.

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[*] "Lancet," 1899, vol. i, p. 370. [†] "Ency. K. d. mik. Technik," p. 785.
[‡] "Med. News," Aug. 8, 1903, p. 248.

Eosinophils display blue or dark-lilac colored nuclei. The granules are stained red by the eosin (Plate 13), but the cytoplasm in which they are imbedded is of a blue color.

Large mononuclears present blue or dark-lilac colored nuclei. Some of these cells show pale-blue or lilac cytoplasm, while others in addition contain dark-lilac or deep-purple granules.

Mast cells resemble the ordinary polynuclear leukocyte, and in addition display coarse spheric granules, which are stained dark-blue, purple, or at times blackish.

Myelocytes contain purplish or dark-blue nuclei. In the cytoplasm are seen numerous dark-lilac or reddish-lilac granules.

Blood-plates appear as small round or oval bodies and are stained blue or purplish. These bodies show irregular margins and their substance contains fine blue or purplish dots.

Preparation of Wright's Stain.—1. Prepare in an Erlenmeyer flask a one-half of one per cent. solution of sodium bicarbonate, and to it add one per cent. of methylene-blue (Grubler "BX," "Koch's," or "Ehrlich's Rectified").

2. Place the mixture in an Arnold steam sterilizer for one hour.

3. Remove and allow to cool; then pour into a large flask and add gradually (agitating after each addition) sufficient quantity of a 1–1000 solution of yellow eosin (Grubler's soluble in water) until the mixture develops a purple color and a yellowish, metallic scum forms on the surface. A finely granular, blackish precipitate is now partially suspended in the mixture. Approximately one part of blue solution to five parts of eosin solution gives this result.

4. Collect the precipitate on a filter-paper, dry thereon, and powder (see *Jenner's Stain*).

5. Place three-tenths of a gram of this dried stain in 100 c.c. of methyl-alcohol.

6. Filter this saturated alcoholic solution of the precipitate, and to the filtrate add 25 per cent. (one-fourth its quantity) of methyl-alcohol.

Caution.—Wright's stain is permanent, and may be kept ready for use. It must be well corked, since evaporation of the alcohol enhances precipitation, in which instance dark particles of the stain are found on the stained blood-film.

Ehrlich's Tricolor Mixture.—Saturated aqueous solution of orange G, 6 c.c.; saturated aqueous solution of acid fuchsin, 4 c.c.; to this is added, a few drops at a time, shaking between each addition, saturated aqueous solution of methyl-green, 6.6 c.c.; then add glycerin, 5 c.c.; absolute alcohol, 10 c.c.; and water, 15 c.c. Shake well for two or three minutes, and allow to stand twenty-four hours. *Do not filter.* Grubler's stains are necessary.

Fig. 39.—Wash-bottle.

This mixture is used daily in my laboratory, and meets clinical demands in every respect. It is well to remember, however, that it deteriorates rapidly, and is therefore not satisfactory in the hands of the inexperienced, and also that it is often troublesome when used by its most ardent advocates. Certain admirable features are most prominent, such as, first, a single staining accomplishes the result; second, it is impossible to overstain the specimen, as overstaining always depends upon faulty technic in the fixing of the specimen (underheating). When films are brought to a temperature of 100° or 120° C., the heating should be continued for a long time. When underheated, the specimen is brown or red instead of orange yellow; when overheated, a pale lemon yellow to the naked eye, and under the microscope, blurred.

Method.—Place the slide or cover-glass in a forceps (Figs. 184–186), and lift a few drops of the stain by means of a pipet, allowing the pipet to pass only a short distance below the surface of the liquid.

Caution.—Never shake the bottle containing the stain nor agitate the solution with the pipet. Cover the entire specimen with the stain, and allow it to remain for five minutes. Remove by washing in water from a wash-bottle (Fig. 39). At this point the specimen is to be dried between blotting-paper and mounted in Canada balsam. It is customary for the author to follow Ehrlich's stain with a 2 per cent. aqueous solution of methylene-blue applied for ten seconds, and removed by washing. The specimen is then dried by heat or between blotting-paper and mounted in balsam. The methylene-blue brings out more clearly the nuclei of the cells.

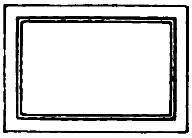


Fig. 40.—Slide label.

Method of Mounting.—The mounting of the specimen which has been prepared upon the slide is accomplished as follows:

1. Drying the specimen thoroughly in the air, by heat, or between filter-paper.
2. Add a drop of balsam to the center of the specimen, care being taken not to cause bubbles.
3. Allow the center of a clean cover-glass to fall directly upon the summit of the drop of balsam, which is spread by the weight of the glass.

The slide may be examined immediately under a low-power objective and, if need be, immersion oil may be added to the center of the cover and a one-twelfth oil-immersion objective brought into focus without destruction to the specimen. *Slides should be immediately labeled* (Fig. 40) and mounts permitted to rest upon their surface for twenty-four hours while the balsam becomes firm; after which they may be rung (see *Method for Ringing*, page 28). Slides containing permanently mounted specimens should be placed in a case (Fig. 41) to rest upon their surface for twenty-four hours.

Fig. 41.—Case for mounted slides.

THE ERYTHROCYTES.

The characteristic features of the erythrocytes in the blood of man are best described by the accompanying plates (2, 6, and 7), and their fidelity as to size, form, and the smoothness of their surfaces is ever to be borne in mind; since deviations from this standard, when not the result of faulty technic, have a definite significance. They vary in number from 4,500,000 to 5,000,000 per cubic millimeter, and 6,000,000 is not an uncommon finding in healthy young men. Menstruation, childbirth, and lactation diminish the number of red cells temporarily, and such diminution depends upon the capability of the individual organism for cell regeneration. The number of cells is low at puberty. Either chronic or temporary dilution of the blood, as may result from the taking of large quantities of liquid, lowers the number of cells, the specific gravity, and the hemoglobin. Hot and cold baths, exerçise, etc., from their effect upon the vasomotor system, may temporarily concentrate or dilute the blood of the peripheral circulation and cause it to show either an increased or diminished number of cellular elements, and the hemoglobin and specific gravity will here be found in direct proportion to the degree of concentration or dilution.

The blood is temporarily diluted after a meal at which liquids have been taken liberally. Fasting produces concentration of the blood and a temporary increase in the number of the red cells. Muscular persons, as a rule, have more red cells than fat individuals. Again, malnutrition diminishes the number of red cells, and exhaustion may also be found to lessen the number of cells in certain cases. Age plays a prominent part as regards the number of red cells—the infant usually shows from 6,000,000 to 8,500,000 erythrocytes per cubic millimeter during the first week of life, while at the other extreme of life the cells may often be found below the normal, yet this is not constant. The influence of season, race, and climate is unimportant in this consideration.

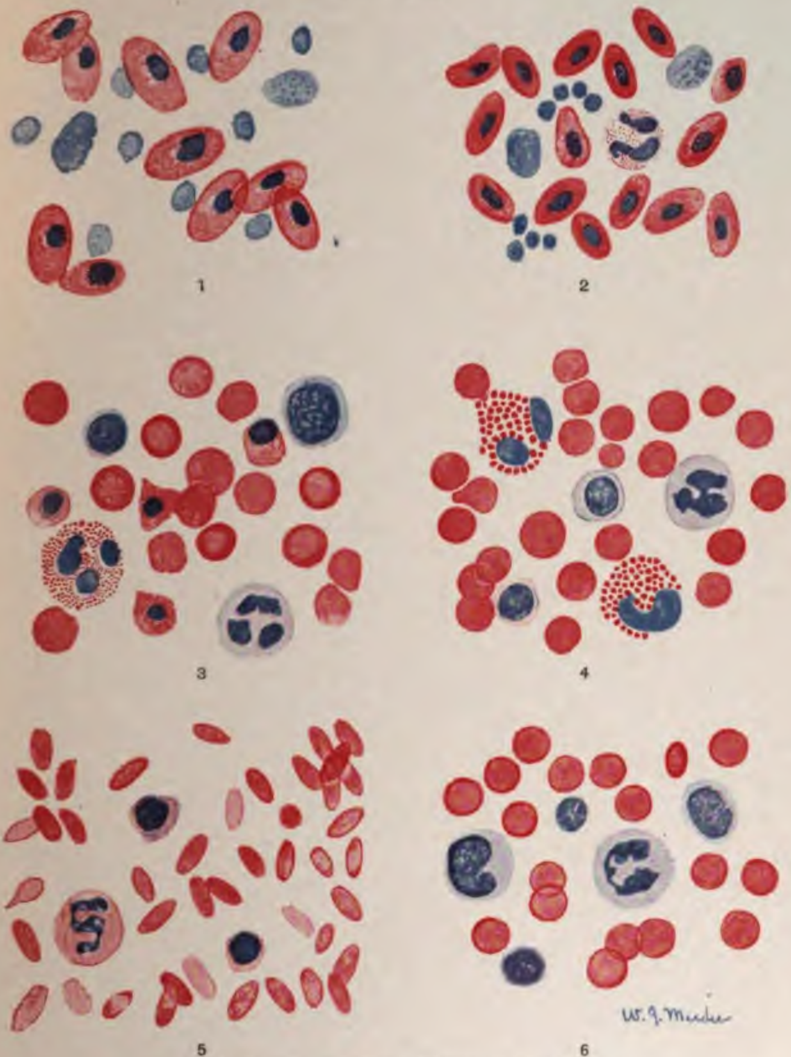
ANEMIA.

Anemia is a condition wherein there is a deficiency in the red cells or in the hemoglobin or in both, with or without change in the number of leukocytes, the total volume of blood, or further appreciable alteration in the chemic composition of either the cellular elements or the serum. For clinical study anemia may be divided into two classes, primary anemia and secondary anemia. In the first class are to be placed conditions where there exists an impoverishment, either of the whole blood or of any of its properties—the cause of such impoverishment being unknown; *e. g.*, pernicious anemia, leukemia, and chlorosis. Secondary anemia includes conditions wherein the blood becomes impoverished as the result of some known cause; *e. g.*, diabetes, organic heart disease, tuberculosis, etc.

Generally speaking, every anemia is secondary, since even in the first variety of our classification there must exist some cause. Sufficient tangible evidence is available, however, to warrant our regarding some forms of anemia as dependent upon a primary disease of the blood-making organs, or upon a specific process resulting in a destruction of one or more elements of the blood. It must be remembered that secondary anemia may simulate closely, and is often not to be distinguished from, primary anemia; *e. g.*, syphilis, gastric carcinoma, intestinal parasites (ankylostomiasis). Anemia identical with pernicious anemia may follow the more severe secondary forms; and, again, primary anemia may result from mental strain, pregnancy, fright, anxiety, and grief, in which cases it is termed "primary" (see ***Individual Forms***), though in reality such anemia is "secondary."

Chemistry of the Erythrocytes.—The analyses of Hoppe-Seyler, Schmidt, and Judell, though subject to criticism since the red cells were separated from the serum by the use of sodium sulphate and sodium chlorid, furnish the best available data for their approximate chemic composition. The specific gravity of the erythrocytes is near 1.088, and they contain nearly 90 per cent. of oxyhemoglobin, a slight amount of globulin-like albumin which coagulates at 75° C., and a trace of cholesterin and lecithin.

PLATE 2.



Blood-corpuscles of Various Animals.

1. Frog's blood: Red cells nucleated, elliptic; white cells small, with indistinct rough margins.

2. Pigeon's blood: Note the fine granules of the eosinophiles.

3. Blood from umbilical cord of the fetus, showing nucleated red cells.

4. Horse's blood: Note contour of red cells and also large granules of the eosinophilic leukocytes, one of which is mononuclear.

5. Camel's blood: Note contour of red cells and irregularity in staining. No nucleated red cells were found. Two animals were studied.

6. Normal human blood. (All specimens stained with eosin, hematoxylin, and methylene-blue. Obj. B and L. one-twelfth oil-immersion.)

Such salts as phosphate of sodium, potassium, calcium, magnesium, and chlorid of potassium are present. The predominant salt found in the blood-serum is NaCl.

Increased Specific Gravity.—The specific gravity of the red cells according to Schmidt is increased in cholera, dysentery, and dropsy; and a chemic analysis shows that they partake of the changes which affect the serum—losing water, salt, and, lastly, albumin.

Recently an attempt at estimating the normal contents of nitrogen for the red cells was made by v. Jaksch, who found wide variations in both health and disease. The dried residue of the red cells after the addition of sodium oxalate has been found by Biernacki* to be equal to from 29.28 to 30 per cent. This percentage has been known to fall in cases of both essential and secondary anemia.

Increased Water.—The increased amount of water in the red cells has been observed in hydremia by the same author, who has contributed valuable data upon the relation of P_2O_5, Fe, K, and also sodium chlorid in the erythrocyte (see ***Hemoglobin***).

HYDREMIA.

The liquid properties of the blood may be found increased in a variety of conditions. A temporary dilution of the blood is seen after the ingestion of large quantities of liquids, hypodermoclysis, enteroclysis, and when large quantities of water have been extracted from the tissue (see *Diabetes*, page 68); but when found in connection with such conditions, hydremia is of limited significance. When the total volume of blood remains nearly constant and there is a loss of solids or of the cellular elements, there must of necessity be an increase in the liquid properties; therefore the blood of anemic persons is hydremic.

Clinical Significance.—The blood of women is usually less rich in corpuscles and it is more hydremic than the blood of men. The blood of persons suffering from secondary anemia and of chlorosis shows about the normal amount of water in their serums. In pathologic conditions accompanied by dropsy the blood may contain more than the normal amount of water, which affects both the corpuscles and the plasma.

ANHYDREMIA.

A reduction of the total volume of blood with concentration of the solids may result from both physiologic and pathologic conditions; *e. g.*, violent exercise with profuse perspiration, and dry diet; or after depletion, as is seen in dysentery, cholera asiatica, during certain stages of diabetes mellitus, and also in plethora. Anhydremic blood may contain an excess of fats.

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* "Zeit. klin. Med.," vol. xxiv, p. 460.

POLYCYTHEMIA.

Polycythemia, a condition wherein the whole blood displays too many red corpuscles per cubic millimeter, is physiologic in the new-born; and when pathologic, may be either general or local When general, blood obtained from any portion of the body discloses this phenomenon, which may exist as a result of disease of the heart, obstruction to the circulation through the lung, congenital deformities of the heart, pressure from tumors, serous effusions, etc. The vessels of a given part of the body may be overcrowded with corpuscles as the result of mechanical appliances,—*e. g.*, application of Esmarch's bandage,—from disease, or from vasomotor disturbance (*local polycythemia*).

Cyanosis.—Cyanosis, resulting from whatever cause, may produce an increase in the number of cells in a given quantity (drop) of blood. A single drop of blood is an expression of the whole capillary circulation; yet capillary blood is richer in corpuscles than is arterial or venous blood. Standards of the normal number of corpuscles per cubic millimeter are estimated from capillary blood. I have seen the number of cells per cubic millimeter reach twice that of the normal under such conditions. Blood collected from the capillary circulation of a paralyzed limb is unusually rich in cellular elements (*local polycythemia*).

In chronic cyanosis the number of red cells will often be found to be from 7,000,000 to 10,000,000 per cubic millimeter, and the hemoglobin is also far above the normal, varying from 120 to 150 per cent. The specific gravity of the blood is likewise increased to from 1.065 to 1.080. The leukocytes are found to be less influenced by general chronic cyanosis, and usually vary from 5000 to 16,000 per cubic millimeter, rarely reaching 20,000. Osler* has reported a series of these cases wherein the maximum findings were red cells 12,000,000, leukocytes 20,000 per cubic millimeter, hemoglobin 165 per cent., and the specific gravity 1.083.

Caution.—Cyanosis figures prominently in all blood counts made upon those suffering from severe anemia, or from such conditions as pneumonia, and where large serous effusions exist. I have repeatedly seen gross error resulting from failure to observe this important factor—cyanosis. In a case of pernicious anemia observed at the Philadelphia Hospital, blood from the lobe of the ear showed 1,840,000 red cells per cubic millimeter, while blood from the finger (hand cyanosed) showed 4,600,000. In lobar pneumonia with extensive consolidation, and in all conditions accompanied by cyanosis, we find the number of corpuscles per cubic millimeter above the normal, when the total blood is really poor in cellular elements.

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* "Amer. Jour. Med. Sci.," Aug., 1903.

After recovery from some prolonged illness, associated with severe anemia, regeneration of the red cells may be rapid and their number reach 6,000,000 per cubic millimeter. In obesity I have seen the red cells reach 6,000,000 to 7,000,000 per cubic millimeter, and, through the courtesy of Dr. J. M. Anders, a similar count was obtained from a boy eighteen years of age, who, formerly a resident of Philadelphia, had just returned from a year's visit to the tropics. The hemoglobin was 114; and the blood became normal after a six months' stay in Philadelphia.

Burns.—Locke, in severe burns with recovery, found an increase in the erythrocytes of from 1,000,000 to 2,000,000 per cubic millimeter to take place within a few hours. An increase of from 2,000,000 to 4,000,000 per cubic millimeter developed in fatal cases.

Anesthesia.—Study of a series of cases reported in collaboration with Dr. J. M. Anders shows that polycythemia results from the administration of ether both in healthy men and in rabbits. These experiments consisted in counting the blood immediately before the administration of the anesthetic, and in making subsequent counts every twenty minutes during the administration of the ether; one hour after the ether had been discontinued, and several times daily until the blood returned to the normal. Here blood concentration was not accompanied by a decided rise in the hemoglobin (see *Hemoglobin*, page 41). In the study of human blood after the administration of ether it should be stated that the patients were not purged before administering the anesthetic; therefore the polycythemia observed depended upon the effects of the anesthetic, as did also the great reduction in the hemoglobin. Polycythemia resulting from the administration of ether was found to be of short duration, and was always followed by a decided reduction in the number of red cells, which reduction followed the fall in the hemoglobin (see *Hemoglobin*).

Causes.—Temporary or permanent lack of fluid within the vessels causes concentration of the blood, and may result from the limited use of liquids, purgation, diarrhea, dysentery, profuse sweating, vomiting, and the rapid accumulation of serous effusions. In a case observed in the Philadelphia Hospital with an extensive pleural effusion of some weeks' duration the red cells numbered 3,860,000. Forty ounces of fluid were removed from the pleura by aspiration, but at the end of twenty-four hours the effusion had reappeared in the pleura and at this time the red cells numbered 5,200,000 per cubic millimeter, an increase of 1,340,000 cells.

Great variation may be found in cases of phthisis when the blood is counted before and after a night-sweat. Thermic changes and drugs (suprarenal extract) may cause temporary concentration of the blood, as does also a rise of the blood pressure. Concentration depends upon interchange of contents between the blood-vessels and the tissues, but this change affects mostly the watery element of the blood; the red cells taking but little, if any, part in the process. Cold induces an increase in the number of leukocytes in the peripheral circulation; yet evidence to show that there is an increase in the total number of leukocytes is wanting. Concentration dependent upon such causes exists but for a short time, since the general body tissues appear to give up sufficient liquid to restore this loss.

Effect of Altitude upon the Blood.—The blood of persons living at high altitudes shows an increase of red cells per cubic millimeter. This change is also manifest in those living in low altitudes who make short sojourns among the mountains. The higher the altitude, the greater the increase in the number of cells. The hemoglobin, too, increases under such conditions, but this increase is not in proportion to that of the red cells. The blood of persons changing from near the sea-level to an altitude of several thousand feet displays great irregularity in the size and shape of the erythrocytes (microcytes) during the first few weeks' stay in such districts, but later these conditions disappear, and the hemoglobin reaches a level corresponding to the number of red cells. After returning from the mountains to the lowlands the blood count rapidly returns to the normal. Herrera and Lope collected data from the literature, and in the following table I have abstracted liberally from their compilation, endeavoring to tabulate the average number of erythrocytes found to be representative of the normal at various altitudes:

LOCATION.	ALTITUDE.		BY WHOM OBSERVED.	RED CELLS PER CENTIMETER.
	METERS.	FEET.		
Christiania	Sea-level	0	Iaoche	4,970,000
Paris	78	255	Hayem	5,000,000
Göttingen	148	485	Schapper	5,225,000
Tübingen	314	1,030	Reinert	5,322,000
Zurich	412	1,351	Stierlin	5,752,000
Auerbach	400–450	1312–1476	Wolf and Koppe	5,748,000
Reiboldsgrün	700	2,296	" " "	5,970,000
Arosa	1800	5,905	Egger	7,000,000
City of Mexico	2256	7,550	Herrera and Lope	6,500,000
Colorado Springs	1824	6,000	Campbell and Hoagland	5,700,000
Morococha	4392	14,409	Viault	8,000,000

A slight increase may result from latitudinal variations. Clinically, we not only observe an increase in the number of red cells and in the density of the blood of persons residing at elevated regions, but hand-in-hand with these phenomena there develop increased density of the urine, milk, body liquids, and diminution of the intravascular tension of the blood. A number of theories have been advanced concerning the production of the polycythemia of altitudes, but it appears plausible that polycythemia and its concomitant phenomena result from diminished barometric pressure, which lessens the amount of oxygen or oxygen pressure. The red cells being the hemoglobin carriers of the body are the first of the body tissues to manifest this change, and their increase in number bears sufficient close relation to the degree of altitude to suggest that the increase is just sufficient to meet the demands made by the general body tissues for oxygen. The time needed to establish this equilibrium is quite short. Campbell and Hoagland* obtained the following counts from healthy individuals living at an altitude of 6000 feet, who were then transported to elevations of 10,000 and 14,147 feet, and returned to the starting-point:

6000 FEET.					10,000 FEET.		14,147 FEET.		6000 FEET.	
Sex	Age	Weight	Pulse	Count	Pulse	Count	Pulse	Count	Pulse	Count
Male	44	189	78	5,760,000	84	6,070,000	84	6,100,000	78	5,790,000
"	27	150	80	6,420,000	104	6,440,000	96	6,510,000	82	5,840,000
"	17	135	80	5,740,000	80	5,700,000	84	5,730,000	78	5,670,000
"	16	150	82	6,150,000	88	6,280,000	80	6,390,000	80	5,970,000
"	19	160	90	6,040,000	84	6,150,000	88	6,240,000	85	6,420,000
"	18	145	60	5,890,000	62	6,750,000	78	6,960,000	60	5,500,000
"	19	120	80	5,680,000	86	6,340,000	92	6,380,000	78	5,575,000
Female	40	123	78	6,120,000	80	6,105,000	88	6,485,000	76	5,700,000
"	51	112	84	5,480,000	88	6,670,000	90	6,170,000	80	5,090,000
"	20	140	84	5,170,000	88	5,790,000	96	5,955,000	82	5,790,000
Average	27	142	80	5,845,000	84	6,229,500	88	6,292,000	78	5,734,500

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* "Amer. Jour. Med. Sci.," vol. cxxii, p 654.

A rise of 50,000 red cells per cubic millimeter followed each additional 1000 feet of altitude.

Polycythemia of Poisons.—Carbonic Oxid and Phosphorus-Poisoning.—After poisoning with CO the number of both red and white cells is increased, but here there is not a drawing away of the liquid properties of the blood. In acute phosphorus-poisoning the number of red cells is often greatly increased, which increase may be explained, in part at least, by the occurrence of profuse vomiting. For description of Polyglobulia see p. 524.

SECONDARY ANEMIA.

Early in the course of secondary anemia there is but slight, if any, diminution in the number of erythrocytes, but as a rule each cell is pale (poor in hemoglobin), and yet all the cells may not share this loss. There is often found an unequal distribution of hemoglobin, certain cells appearing pale, while others are normal, of small size, and light in weight, as shown by a low specific gravity. Such anemia commonly results from unhygienic surroundings. As the condition progresses there is to be observed merely an exaggeration of the changes above mentioned, and in addition many of the cells become deformed and irregular in size, varying from that of mere dwarfed cells to giant cells, many of which display great irregularity of outline (poikilocytes). The following text in conjunction with plate 3 will explain the characteristic features of the various abnormal erythrocytes. The several degenerative changes to which the red cell is subject may be classified as follows:

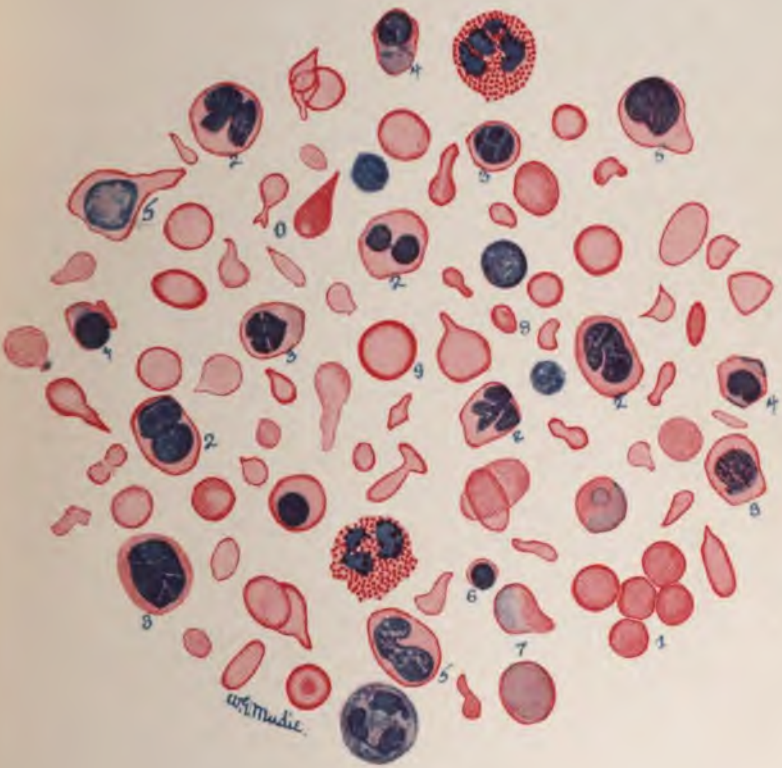
I. Alterations in viscosity.
II. Endoglobular changes or simple decoloration.
III. Changes in the shape, size, and motility.
IV. Atypical staining properties.
V. Granular basophilia.
VI. Total necrosis and disintegration.
VII. Megaloblastic forms.

Many of these changes in the erythrocytes may be seen to take place outside the body; and under pathologic conditions there are to be found cells displaying such abnormalities.

Viscosity.—This property of the blood is detected by collecting fresh blood upon a slide and permitting it to be exposed to the air for a short time, when with advancing coagulation the greasy, slippery feel of fresh blood is replaced by a varying degree of tenacity or stickiness (viscosity).

Rouleaux Formation.—Should the viscosity be increased (*hyperviscosity*), the erythrocytes instead of forming rouleaux—when the red corpuscles agglutinate one upon another like a

PLATE 3.



VARIOUS FORMS OF ERYTHROCYTES.

1, Normal erythrocytes; 2, karyokinetic changes in the nuclei of erythrocytes; 3, pigmentation of the nuclei of erythrocytes; 4, polychromatophilia in nucleated erythrocytes; 5, megaloblasts; 6, microblast; 7, polychromatophilia of macrocytes; 8, microcytes; 9, macrocytes; 10, poikilocytes. At the lower margin of the picture is seen a basophilic leukocyte. (From blood of a child studied at Pennsylvania Hospital. Obj. B. and L. one-twelfth oil-immersion).

column of coins—appear in dense irregular aggregations, and when a cell is found separated from these masses, it may be greatly distorted. When the viscosity is greatly diminished (*hypoviscosity*), the erythrocytes are found singly, floating free in the plasma, and showing little or no tendency to rouleaux formation. The viscosity of the blood is influenced largely by the erythrocytes, yet the viscosity of the serum is a factor of importance.

Estimation.—The viscosity of human blood is estimated at about five times that of distilled water. The viscosity of a blood having a specific gravity of from 1.045 to 1.055 is expressed by the figure 5.1, which is in comparison with that of water (1). To determine this point both fluids must be at a temperature of 38° C. A close relationship between the degree of viscosity and the specific gravity does not exist, yet collectively speaking the lower the specific gravity of the blood, the less marked its adhesiveness.

Clinical Significance.—The viscosity of the blood is increased by a diet largely nitrogenous, exposure to the action of snake venom,* and by contaminating the fresh blood with serum from patients suffering from chlorosis, leukemia, or pernicious anemia (Stengel). In the severe anemias the non-adhesiveness of the erythrocytes is apparent.

Endoglobular Changes (Simple Decoloration).—Decoloration of the erythrocytes is a most common feature of the blood in mild anemia when there is but slight involvement of either the hemoglobin or the cellular elements. Decoloration begins as a symmetric enlargement of the normally lighter area at the center of the cell and progresses toward the periphery. Such cells soon lose their normal biconcavity, and where decoloration progresses the cell is converted into a mere shell whose margin shows a faint color due to the persistence of hemoglobin (*achromacyte* or *shadow cell*). Areas of pallor may be distributed throughout the cell when there is a dissemination of the degenerative process; such areas are of various forms and sizes, outlining the stroma of the cell from which the hemoglobin has been removed. Areas of pallor in the erythrocyte have a selective affinity for basic dyes: *e. g.*, thionin, by which they are brilliantly stained (see *Basophilia*, page 91). Normally, these changes occur in from thirty minutes to an hour after the blood has been withdrawn from the vessels, but in pathologic conditions they may be seen immediately after the blood is withdrawn, and it is supposed that they exist within the vessels.

Changes in Shape, Size, and Motility.—Poikilocytosis and **crenation** consist of decided irregularity in the form of the erythrocytes. Crenation is produced by a portion of the cell becoming looped and one or more points projecting from it (serrated margin). These points may become prominent, and in time the entire cell assumes ameboid movements. Such movements when they occur in rapid succession are responsible for the various shapes of the corpuscles (*poikilocyte*, see Plate 3). The *ameboid motion* of the erythrocytes has been studied at length by Hayem, but does not bear sufficient clinical significance to warrant description in this chapter. It may cause portions of the cell protoplasm to break from the corpuscle (*schistocyte*), and these particles after floating in the plasma for a time become spheric and resemble miniature erythrocytes (*dwarf-cells*).

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* Weir Mitchell, "N. Y. Med. Jour.," vol. vi, p. 289.

Brownian movement, a peculiar vibration of the protoplasm, which is a feature of healthy blood, should not be confused with the movements resulting from crenation. In malaria there is also a Brownian movement of the pigment. In pernicious anemia there is a tendency for the red cells to become oval or elliptic, and poikilocytosis is also pronounced, but oval cells may be found in small numbers in the normal blood, while in any form of anemia they are increased.

Caution.—When deformity in the cells depends upon faulty technic, the spicules extend from the same side of the cells or they point in one direction.

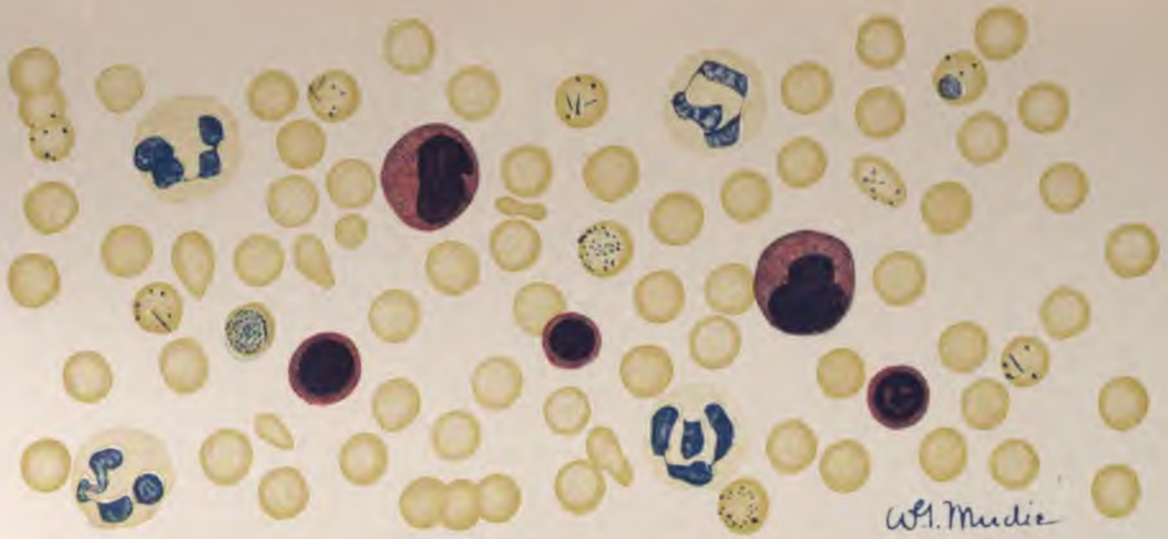
The **megalocyte or macrocyte** (Plate 3) is an extremely large cell, between 12 and 20 μ in diameter, and is the immediate successor of the megaloblast after its nucleus has been absorbed. Its stroma is usually deficient in hemoglobin (Plate 3) and is likely to possess polychromatic properties. This giantism of the erythrocyte may be the result of imbibition of fluid from the plasma, as is suggested by its dropsical appearance. Another theory promulgated is that its antecedent was a megaloblast of exceptional size.

Clinical Significance.—Macrocytes are found in the blood of secondary anemia, where megaloblasts are conspicuously absent. They are to be found in blood showing megaloblasts. (Plate 6.)

The **microcyte** is a spheric erythrocyte from 3 to 5 μ in diameter, having a deeply colored stroma. The smaller of these cells are known as "Eichhorst's corpuscles." They are of disputable clinical significance.

Atypical Staining Properties.—The normal erythrocytes have decided affinity for acid stains, but changes capable of producing alterations in the form and size of the cells probably cause certain staining abnormalities. Portions of the protoplasm may

PLATE 4.



BLOOD FROM A CASE OF CHRONIC LEAD-POISONING.

(Stained with carbol-thionin.)

Many of the erythrocytes show punctate basic degeneration, one of them being nucleated Decided variation in the size and shape of erythrocytes is observed. There are four polymorphonuclear leukocytes, two myelocytes, and three lymphocytes.

at times take the basic dyes (*punctate basic degeneration*)—as is exemplified in the blood of lead workers (Plate 4). Again, the cells may be colored by two, three, or more stains that may be contained in the staining mixture. This condition is known as *polychromatophilia;* its causal factors are undetermined, and clinically it points to a high grade of anemia. Certain sections of the cell may stain deeply, while the remaining protoplasm is but feebly stained, and at times the entire cell merely stains as a capsule or ring, while its center is clear; or, the cell may have little or no hemoglobin, and appear as a shadow with an indistinct margin (*shadow cell*).

Cabot's Ring-bodies.—Cabot* has described ring-shaped bodies in the red cells which, when stained by eosinate of methylene-blue dissolved in methyl-alcohol, appear bright red in contrast to the surrounding protoplasm, which is pale yellow. These bodies occur in lead-poisoning, pernicious anemia, and lymphatic leukemia. Their clinical significance remains questionable.

Granular basophilia or "punctate basic degeneration" is a condition characterized by small areas of degeneration disseminated throughout the protoplasm of the erythrocyte. In the stained specimen they are colored by the basic dyes (thionin, methylene-blue, and hematoxylin). After staining with a solution of carbolated thionin for five minutes they become quite conspicuous when studied under a one-twelfth oil-immersion objective. They commonly assume the character of small amorphous or oval granules, though short rods and spicules are also to be seen (Plate 4).

Clinical Significance.—Basic degeneration is to be observed in most high grades of anemia, but is found abundant in the blood of lead workers.† Judging from current literature, there is sufficient evidence to show that these granules are not the product of nuclear fragmentation or of débris. The observers referred to have demonstrated in granular erythroblasts signs of active karyomitosis. These granules are to be found normally in the blood of embryonic mice,‡ and cats; also in developed rabbits,§ squirrels, and guinea-pigs. Personally, I have observed it in the blood of lead workers,|| pernicious anemia, pulmonary tuberculosis, and malaria. Stengel observed these granules in the blood of eleven out of eighteen chlorotics examined.

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* "Jour. Med. Research," Jan., 1903.

† Stengel, White, and Pepper, "Amer. Jour. Med. Sci.," Sept., 1900; also 1902, p. 873.

‡ Pappenheim, "Inaug. Dissert.," Berlin, 1895.

§ Ehrlich, "Die Anämie," Wien, 1900.

|| "Phila. Med. Jour.," Sept. 27, 1902.

Necrosis.—Erythrocytic necrosis and disintegration are caused by exposure of normal blood to the air for from three to four hours. In pathologic blood such changes may be due to an increased toxicity of the plasma or to a lowered cell resistance,—in which instances the red cells are highly susceptible to injury by the plasma,—or by extraneous diversion. These changes may be observed in pathologic blood immediately after it is withdrawn, and are significant of a more severe type of anemia than is simple decoloration (Plate 6).

Any or all of the above varieties of erythrocytes may be found in a single case of secondary anemia, and the frequency with which such cells are to be found is governed by the severity of the existing condition. The third stage of secondary anemia presents, in addition, a slight and, at times, rather marked decrease in the number of the red cells per cubic millimeter. In the fourth or last stage of this condition we have, in addition to the other phenomena manifest, the presence of nucleated red cells, of which there are three varieties, normoblasts, megaloblasts, and microblasts. Nucleated erythrocytes are normal in the blood of reptiles, birds, and in the umbilical cord of the human fetus (see Plate 2).

Megaloblasts.—The megaloblast is an abnormally large nucleated erythrocyte which is not found in the blood of healthy adults nor in the bone-marrow, and is a direct antecedent of the megalocyte, into which it is transformed by the absorption of its nucleus. It is often present in the marrow of the fetus and in the marrow from severe forms of anemia. Ehrlich was the first to describe this cell as characteristic of the fetal type of anemia.

Clinical Significance.—The presence of megaloblasts in the blood may be regarded of serious prognosis, and a sign of degeneration of the hematopoietic organs, including defective hemogenesis of embryonal nature; though not of necessity does a fatal issue ensue. Megaloblasts appear in the blood of the severer forms of secondary anemia, especially that produced by the ankylostoma (*Uncinaria duodenale*), the bothriocephalus, and nitrobenzol poisoning, when they constitute a large percentage of the nucleated red cells. They are fairly characteristic of pernicious anemia. I have also seen them present in the blood after severe puerperal hemorrhage; and nucleated red cells whose size is but slightly above that of the normoblast are not an uncommon finding in anemia.

Description.—The typical megaloblast varies from 11 to 20 μ in diameter. It frequently shows evidence of degeneration in its protoplasm, as swelling, unevenness, flaws, and cracks which do not stain deeply (see Plate 3). The nucleus is, as a rule, large, occupying the greater portion of the cell, and, in striking contrast to the nucleus of the normoblast, it generally stains pale but evenly throughout (see Plate 3). Exceptional cells are to be found, and in the blood of a patient seen at the Pennsylvania Hospital, through the courtesy of Dr. J. Allison Scott, many uncommon forms of megaloblasts were represented (see Plate 3). Surrounding the nucleus there is often a clear narrow ring outside of which the protoplasm may stain rather deeply, while toward the margin of the cell the protoplasm, at times, presents a purplish tint (*polychromatophilia*).

In the blood just referred to it was very difficult to separate the various forms of nucleated erythrocytes and, in fact, there appeared to be no one cell predominant, while there was a great variety of atypical cells, as shown in plate 3. The experience of hematologists shows that under all conditions megaloblasts cannot be distinguished from normoblasts by any one or by any methods; and that border-line cells are at times equally numerous with those characteristic of either type. Pappenheim regards the structure of the nuclear net-work as the most valuable differential point, but this is often difficult to detect. The majority of megaloblasts are young cells displaying large pale nuclei, and the bulk of the normoblasts older cells, presenting smaller nuclei, which stain more deeply. These features appear to be sufficient for practical differentiation. From a clinical point of view a most striking feature is that of a child whose blood presented these changes and who made an uneventful recovery (see Plate 3)—infantile anemia.

The **normoblast** is a nucleated red cell which corresponds in size to that of the normal red cell, is conceded to be its antecedent, and is to be found in the bone-marrow of healthy individuals and more plentifully after great depletion of the body fluids. It is regarded as the younger stage in the life of the red corpuscle, and is rarely seen in the healthy peripheral blood.

Clinical Significance.—The appearance of normoblasts in the peripheral blood is suggestive of a decided production of the normal erythrocytes by the marrow, and that in this attempt to supply the demand made upon the marrow, certain cells are forced into the circulation before they are fully matured and have extruded their nuclei. Plate 6 will illustrate the various forms of normoblasts. Free nuclei are occasional findings. The nucleus may be stained evenly throughout—pale, dark, or present small, highly refractile dots. Two nuclei are usually connected by a feeble cable. Rarely the nucleus is located upon the margin of the cell (Plate 5); again, partially extruded; and when completely within the cell, may assume almost any shape.

The **microblast** is a small erythrocyte whose nucleus is less liable to variation than is that of the normoblast (Plate 3). The nucleus stains deeply and occupies the greater portion of the cell, leaving a thin band of protoplasm surrounding it.

Oligemia (**Quantitative Anemia**).—Oligemia signifies a reduction in the total quantity of the blood. It usually results from extensive hemorrhage, and is temporary, since water is extracted from the tissues to restore the total volume of blood to that of the normal. During the existence of oligemia the vessels contract, and thus accommodate themselves to the lessened quantity of blood.

Melanemia.—Melanemia is a condition in which dark pigment-granules appear in the circulating blood, and is noted in the blood of malaria and rarely in that of other febrile conditions. The yellowish or blackish granules of pigment are seen in the plasma or in the bodies of the leukocytes.

NORMAL LEUKOCYTES.

The figure commonly accepted for the healthy adult is 7500 cells per cubic millimeter, but this will be found to vary greatly in such conditions as malnutrition, time of day, exercise, influence of digestion, hot or cold baths, etc. Other conditions influencing the number of leukocytes will be considered under the special headings of *leukocytosis* or *leukopenia.*

The terminology here becomes very confusing, and has resulted partly from the theory of the origin of the cell ("lymphocyte"), partly from the characteristics of the "nucleus" ("polymorphonuclear"), and, again, from the affinity displayed for certain anilin dyes ("eosinophilic," "neutrophilic"). The percentage of the various forms of leukocytes will be found to deviate moderately in the healthy adult from the following table:

Two sources are now accepted:

(A) The myelogenic group (from the bone-marrow)	1. Polymorphonuclear neutrophils. 2. Eosinophils. 3. Large mononuclears (Ehrlich). 4. Mast cells.
(B) The lymphogenous group (from adenoid tissues)	Lymphocytes, large and small.

Small lymphocytes	20	to 30	per cent.
Large lymphocytes	4	" 8	" "
Polymorphonuclear neutrophils	62	" 70	" "
Eosinophils	0.5	" 4	" "
Mast cells	0.025	" 0.5	" "

Infants show a high percentage of lymphocytes (50 per cent. or more), while the polymorphonuclear elements are correspondingly reduced in number to from 20 to 40 per cent.

Composition of the Leukocytes.—All efforts to ascertain the chemic composition of the leukocytes have been made by using pathologic blood (leukemic), from lymphoid tissue, or from pus, and it must be admitted that the chemic analyses of leukocytes obtained from such sources are subject to variation. Lilienfeld,* in his analysis of lymphoid tissue, probably gives us the most reliable data. Pus, which but little concerns us in this chapter, has been found to contain a number of different forms of albumin, including Bence-Jones' albumose. In leukemic blood recovered from the cadaver are to be found fatty acids, lecithin in excess, and glycogen. Nucleins are a variety of bodies which may be obtained either from animal or vegetable cells after digestion with pepsin. These are fairly insoluble in alcohol, water, and ether; they give the biuret and Millon's reactions (see *Urine*, page 217). They hold an abundance of phosphorus and iron, and their tinctorial properties facilitate in distinguishing them from the basic albumins; when boiled with dilute acids, give nuclein bases; and when treated with alkalis, albumin and nucleic acid result.

Lymphocytes.—These cells are generally divided into two classes, large and small (Plate 9). The first variety ranges in size from slightly below to moderately above that of the normal red cell, and the greater portion of its body is occupied by a rather compact, often coarsely reticulated, spheric nucleus, surrounded by a narrow band of strongly basophilic protoplasm, which is at times homogeneous and again reticulated. Protoplasmic granules are not present.

The large forms, which vary in size from above that of the normal erythrocyte to that of the polymorphonuclear leukocyte, will be found to possess in a general way features characteristic of the smaller form. At times, however, the reticulum of these cells shows nodular thickenings often resembling protoplasmic granules. The nucleus may show faint rings, suggestive of nucleoli, and nodular thickenings. A spheric nucleus is common to normal blood, but in pathologic conditions strongly basophilic and also hyaline lymphocytes are to be found, certain of which present indented or subdivided nuclei (see *Leukemia*, page 129).

Large Mononuclears.—This cell is but slightly above the size of the large lymphocyte, yet occasionally it is noted to be the largest cell of normal blood. The protoplasm is mildly basophilic, finely reticular, displaying nodular thickenings or questionable granules. Its nucleus is vesicular, showing granular thickening in spots and granules resembling a central nucleolus. The nucleus may be circular, horseshoe-like (*transitional leukocyte*), or, less often, elongated (Plate 8).

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* "Zeit. f. phys. Chem.," vol. xviii, p. 473; also vol. xx, p. 155.

Polynuclear Forms.—This variety of leukocyte is, as a rule, about two or three times the diameter of a normal red cell. Its protoplasm is distinctly reticulated, displaying at various points of the reticulum many neutrophilic granules. As a rule, the protoplasm is mildly basophilic, displaying nodular thickenings here and there when stained with methylene-blue (Plate 5). The nucleus is elongated, constricted, and often distorted so as to form three or more rather distinct lobules, which upon careful study are found to be connected by narrow bridges or threads of nuclear substance. These more or less isolated portions of the nucleus are often slightly granulated, displaying one or more central nodular thickenings, and rarely, indeed, are the various portions of the nucleus entirely separated. To this variety of cell the terms *polynuclear*, *polymorphonuclear*, and *polymorphonuclear neutrophils* are applied. Occasionally, cells are to be seen which display a decided affinity for basophilic dyes,—"*polynuclear basophils*" (see Plate 6),—and these are probably a direct descendant of the first embryonal leukocytes, as shown by a study of fetal blood.

Mast Cell.—This is a coarsely granular basophilic cell which deserves special mention (Plate 7). It is supposed to have its origin in the lymphoid tissue.

Eosinophil.—A form of leukocyte varying in size from a small lymphocyte to the polynuclear forms. Its distinctive feature is that its protoplasm is more or less thickly studded with strongly acidophilic (red) granules. The nucleus is rather coarsely granular, displaying two or more lobes, and, as a rule, stains feebly with the basic dyes (Plates 7, 8). It is not uncommon to find the cell-wall ruptured and numerous red granules scattered about the pale nucleus as though the result of an explosion (Plate 6).

LEUKOCYTES IN DISEASE.

An absolute and relative increase in the number of leukocytes (leukocytosis); an increase in a certain variety of the cells, with or without any marked change in other forms; an absence or a great diminution in the number of any variety of cell, with or without a relative increase in the total number of cells; a decrease in the total number of cells (leukopenia), and the addition of a new cell, the "myelocyte," of which there are two forms,—"myelocyte and eosinophilic myelocyte,"—constitute the relative and morphologic deviations of the leukocyte induced by disease. Changes in both the relative proportion and in the number of leukocytes are caused by conditions on a par with those capable of exciting changes in the erythrocytes, and will be described in detail under the special headings of Leukocytosis and Leukopenia.

Essential Feature.—A conception of the normal relation the different cells bear one to the other, and the recognition of the myelocyte, are all that are necessary to distinguish between the leukocytes of pathologic and those of physiologic blood.

Myelocytes.—The myelocyte, though it differs widely from any other form of leukocyte found in normal blood obtained from the peripheral circulation, resembles in certain respects cells which physiologically are present. It is not characteristic of any one form of disease, and may be occasionally found in the blood from a variety of maladies which are on the border line, so to speak, between pathologic and physiologic states. The myelocyte (Plates 7 and 8) with Ehrlich's triple stain appears as a spheric cell whose protoplasm is nearly occupied by a pale-stained nucleus studded with neutrophilic granules.

Great variation in the staining affinity of the different granules in the same cell is often seen, and "there is prominent a basophilic portion which becomes less and less marked as the cell grows older" (Cabot). Such basophilic properties are seen to be less marked in the polynuclear neutrophilic cells, yet even here considerable difference may be observed in the contiguous cells of the same field, and also of the adjacent granules of the same cell. The size and form of myelocytic granules are not unlike those found in polynuclear cells. Most important as a distinguishing feature of the myelocyte is its nucleus, which is single, and does not display the distortion characteristic of the polymorphonuclear neutrophils; but, on the contrary, is always spheric or egg-shaped, and in almost immediate contact with the cell-wall for a comparatively large portion of its circumference. The spheric nucleus is commonly placed toward one side of the cell. It is necessary to distinguish the myelocyte from the large lymphocyte, which can be said to differ from it only in that it (the lymphocyte) is devoid of granules.

Eosinophilic Myelocyte.—The eosinophilic myelocyte differs from the one described in containing eosinophilic instead of neutrophilic granules. Both this cell and the neutrophilic myelocyte are to be found in great numbers in the bone-marrow. They are likely to display variations in the staining of their granules, the nucleus to show the evidences of old age (vacuoles or mitosis, Fig. 47), since occasionally a cell is seen containing two nuclei. There is no other leukocyte capable of such great variation in size as is the myelocyte, which may vary in diameter from 10 to 20 μ. This variation extends from far above the diameter of the polymorphonuclear leukocyte to that of the lymphocyte.

Clinical Significance.—Myelocytes are found in the blood of splenomedullary leukemia, lymphatic leukemia, after certain severe forms of intoxication, prolonged fasting, essential anemia, grave secondary anemia, and in a number of border-line conditions. I have found them after prolonged ether anesthesia.

Transitional Neutrophils.—These are neutrophilic cells which appear to occupy a position between a true marrow cell (myelocyte) and the polymorphonuclear leukocyte, and display a nucleus which shares certain characteristics common to each of these varieties. Their significance is undetermined.

"Türck's Stimulating Form."—A variety of cell regarded differently by various writers. Weil describes it as resembling a myelocyte whose granules have been fused into a smooth, homogeneous color which surrounds the nucleus. The protoplasm always stains deeply, appears homogeneous; yet there is considerable variation in the tint in different cells, ranging from purple to violet or brown. In the majority of our observations these cells are regarded as either myelocytes or as large lymphocytes, and are not uncommonly referred to as border-cells. Their presence usually accompanies diseases showing leukocytosis.

Degenerated Leukocytes.—This form of cell is most commonly met with in the blood of leukemia, yet it may be found elsewhere. The first impression received is that they are artefacts—abnormalities in cell structure due to faulty preparation of specimen; but this can usually be disproved, since they do not occur in the blood of healthy individuals treated in the same manner. Degenerated leukocytes stain as a homogeneous mass, are structureless, appear to have lost their protoplasm, and their edges are ragged (karyolysis). Vacuolization of both the protoplasm and of the nucleus are not uncommon findings; while the large mononuclear cells display such degenerative changes as breaking up, pallor of the nucleus, structurelessness, and may appear as shadow cells.

DIFFERENTIAL COUNTING OF THE LEUKOCYTES.

The object here is to determine what percentage of the total number of leukocytes present belongs to each of the subvarieties of these cells previously described (page 94). In order that a fair estimation be gained, at least 600 leukocytes should be counted. This examination consists in classifying the leukocytes under their respective heads. It is this class of work alone which makes the mechanical stage most valuable; yet it cannot be said that this accessory to the microscope is necessary; though the use of such an instrument facilitates the making of a differential leukocytic count, and I believe adds materially to the authenticity of the result (count).

Method for Counting.—Bring a properly stained and mounted specimen into focus under an oil-immersion objective, and place at the right hand the following table, upon which to record each cell under its respective class. The slide is carefully moved until we have covered the surface of the smear, or at least sufficient of the surface to have counted in all 600 leukocytes.

DIFFERENTIAL LEUKOCYTE COUNT.

Name ....

Residence ....

Polymorphonuclear neutrophils	408	=	68.0	per cent.
Large lymphocytes	30	=	5.0	" "
Small lymphocytes	108	=	18.0	" "
Large mononuclears	18	=	3.0	" "
Mast cells	3	=	0.5	" "
Eosinophils	15	=	2.5	" "
Myelocytes	12	=	2.0	" "
Eosinophilic myelocytes	6	=	1.0	" "
Total	600	=	100.0	" "

During the counting of 600 leukocytes there are found—

Nucleated red cells	Normoblasts	4
	Microblasts	3
	Megaloblasts	3

The estimation of the percentage of each variety of cells present in this given specimen of blood is accomplished by using the number of each variety of cell found as the one factor, and 600, the number of cells counted, as the other.

Nucleated Red Cells per Cubic Millimeter.—The number of nucleated red cells seen while counting 600 leukocytes is also recorded (see above); and the number per cubic millimeter is readily estimated in the following manner:

Let a = number leukocytes counted, or 600
" b = number nucleated red cells seen, or 10
" c = number leukocytes per cubic millimeter, or 12,000

$c \text{ or } 12{,}000 \times \frac{b \text{ or } 10}{a \text{ or } 600} = 200$, the number of nucleated red cells in one c.mm. of undiluted blood.

Counting.—In counting begin at the upper left-hand corner of the specimen, and cause the lens to traverse across the specimen from top to bottom, moving the slide a sufficient distance to the right after each crossing of the slide so as not to include any of the cells which have been previously counted (Fig. 32). It requires but little practice until one becomes able successfully to manipulate the slide. The time necessary for making a differential count depends entirely upon the number of leukocytes found upon the smear, and when the leukocyte count is below 12,000, the procedure is tedious.

BLOOD-PLATES.

Blood-plates (Plate 13) appear as cohesive, circular or ovoid, homogeneous bodies with irregular outlines, and are from one-third to one-half the diameter of the erythrocyte. They are devoid of hemoglobin and of nuclei, and are rarely seen in normal blood. Their number has been estimated by von Emden at from 180,000 to 250,000; Pruss gives the average as 500,000; and Kemp and Calhoun, 860,000 per cubic millimeter. Blood-plates form a prominent constituent of white thrombi, and whenever they are diminished, clot-formation is apt to be retarded, and the possibility of hemophilia or purpura may be suspected; also a lowered resistance of the erythrocytes. An increase in the number of blood-plates is suggestive of the development of thrombi.

Clinical Significance.—In pernicious anemia they are diminished and thrombosis is comparatively rare. An increase occurs in leukemia after hemorrhage, and in certain other grave afebrile anemias, and they are normal or increased in tuberculosis and pneumonia. In such types of infection as erysipelas, malaria, and typhus, and in purpura and hemophilia, they are diminished, and in the latter condition at times absent. It should be borne in mind that only intravenous thrombosis is influenced by blood-plates, and that clot-formation outside the body is an entirely different phenomenon, depending upon the amount of fibrin present in the individual's blood. Opinions differ as to the origin of blood-plates.

Chemic Composition of Blood-plates.—Löwit maintains that blood-plates are composed of globulin; while Lilienfeld believes them to belong to the nucleo-albumins, and suggests that they result from the destruction of the nuclei of leukocytes.

BLOOD DUST.

Blood dust—"hemokonia"—is a constituent of both normal and pathologic blood, which has until recently received but little attention. It appears as small, round, colorless granules, which correspond in size and form to fine oil-droplets; their size ranges from one-fourth to one micromillimeter in diameter. These granules are highly refractile bodies, displaying a rather rapid dancing motion, and are devoid of locomotion. Blood dust is insoluble in alcohol and in ether; does not stain by osmic acid, and has not been shown to contribute toward the formation of fibrin. The light of the Welsbach burner appears to assist in studying these bodies, which are also to be found in pus and in the fluid from the serous sacs.

LEUKOCYTOSIS.

Leukocytosis is a condition characterized by an increase in the number of leukocytes in the peripheral circulation over the number commonly found in the same individual, without a diminution in the number of polymorphonuclear cells, but usually this variety shows both an absolute and relative increase over the number normally present.

Physiologic leukocytosis appears some hours after the ingestion of a meal rich in carbohydrates, and after exercise, massage, cold baths, parturition, during pregnancy, and in the new-born.

Pathologic leukocytosis follows hemorrhage, and in certain infectious diseases, as relapsing fever, Asiatic cholera, erysipelas, epidemic cerebrospinal meninigitis, pneumonia, bubonic plague, yellow fever (not constant), glanders, actinomycosis, acute articular rheumatism, scarlet fever, small-pox, diphtheria, follicular tonsillitis, syphilis (secondary stage), trichinosis (usually moderate), filariasis (early), malignant endocarditis, acute pancreatitis, gonorrhea, acute endometritis, cystitis (occasionally), acute pyemia, septicemia, cholangitis, cholecystitis, and shortly before death (terminal leukocytosis). It is seen in a variable degree in connection with inflammatory processes involving either the deeper tissues, viscera, or skin, as well as in certain forms of malignancy, lithemia, gout, toxemias and in lead workers where they fluctuate between 9000 and 25,000 during the primary attack of colic.*

Among the more strictly localized processes that may cause leukocytosis are abscesses involving either the glands or other tissues, appendicitis, osteomyelitis, pyelonephritis and pyelonephrosis, perinephritic abscess, abscesses involving any of the viscera, localized peritonitis, epididymitis, conjunctivitis, proctitis, and any acute inflammation of the serous membranes. Leukocytosis is also a component feature of gangrenous stomatitis, noma, etc., and is often well marked in such cutaneous conditions as pemphigus, herpes zoster, eczema, dermatitis, urticaria, pellagra, ichthyosis, and, rarely, in psoriasis.

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* Boston, "Phila. Med. Journal," Sept. 27, 1902.

Toxic Leukocytosis.—For want of a better term the following conditions have been arranged under this head, which, though indefinite, explains in a measure, at least, the cause of leukocytosis in many of the conditions here grouped; yet these conditions are not always associated with leukocytosis—viz., acute yellow atrophy (hepatic), cirrhosis of the liver with jaundice, uremia, rickets, gout, ptomain poisoning, cinchonism, after intravenous injection of normal salt solution, illuminating-gas poisoning (Cabot), chloroform anesthesia, ether anesthesia, and acute maniacal delirium.

Leukocytosis of Malignancy.—There appears to be some difference of opinion as to the true nature of the leukocytosis of malignant disease. Alexander* found the leukocytes to vary from 2360 to 21,700 per cubic millimeter in cases of malignancy of the breast, and not dissimilar results have been obtained by a number of observers in carcinoma of the stomach and of the rectum. Schneider found leukocytosis in 12 cases of malignancy. Osterspey† detected leukocytosis in but 2 out of 12 cases examined. My own experience suggests that leukocytosis varies with the degree and stage of malignancy, also with its location, and with the presence or absence of ulceration and suppurative surfaces. The wide variation found in over 60 leukocyte counts made upon cases of malignancy renders it impracticable for me to draw any definite conclusions.

Leukocytosis of Anesthesia.—T. L. Chadbourne,‡ in the study of 21 cases, found that there was always an increase of the leukocytes, resulting from the administration of ether anesthesia. Counts were made immediately before the anesthetic was begun and a second count taken after the ether had been continued for sixteen and two-third minutes. The average increase in the number of leukocytes was found to be 37.3 per cent. (the lowest 6, the highest 73 per cent.). Differential counts were made upon 5 of these cases and showed that the increase affected the polymorphonuclear cells and the lymphocytes equally. A fact to be borne in mind is that each case presented some pathologic condition, and the majority of the cases showed over 10,000 leukocytes per cubic millimeter before anesthesia. Operation often appeared to cause a reduction in the number of leukocytes.

A. von Lerber§ counted the leukocytes in 101 cases, and found them to be increased after ether anesthesia in 96 instances. The remaining 5 cases should not be included, since a high-grade leukocytosis existed before anesthesia. In 35 instances the number of leukocytes was double and in 23 triple the number found before ether. The effect of the operation is not considered in his estimation. Blake and Hubbard* studied 28 cases, making counts before and after ether anesthesia, and found the peripheral circulation to show no increase in the number of leukocytes.

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* "Thèse de Paris," 1887.

† "Inaug. Dissert.," Berlin, 1892.

‡ "Phila. Med. Jour.," Feb., 1899, p. 390.

§ "Inaug. Dissert.," Berne, 1896.

I secured permission from a healthy male, aged thirty-three years, to conduct the following experiments upon him. He had never taken ether as an anesthetic, always enjoyed health, and had abstained from alcohol. He was not prepared for the anesthetic by purgation (studied with Dr. J. M. Anders):

Six hours after a meal and immediately before beginning the anesthetic, I found the hemoglobin to be 86 per cent.; red cells 4,080,000; leukocytes 7600 per cubic millimeter. Upon staining, the blood was normal. After ether had been given twenty-five minutes (complete anesthesia, ten minutes) it was discontinued—hemoglobin 79 per cent., red cells 6,150,000, white cells 16,000. Microcytes numerous, all cells rather pale. One hour after ether had been withdrawn, hemoglobin 74 per cent., red cells 4,170,000, white cells 9800. Many of the red cells stained as mere shadows, and their size varied. A differential count of 600 leukocytes gave:

Polymorphonuclear	81.07	per cent.
Small lymphocytes	2.22	" "
Large mononuclear	1.11	" "
Transitional	12.00	" "
Eosinophils	0.38	" "
Myelocytes	4.32	" "

Twenty-four hours after ether (but little vomiting had occurred), hemoglobin 67 per cent. (loss of 19 per cent.), red cells 4,576,600, white cells 13,600.

In collaboration with Dr. J. M. Anders I also conducted a series of experiments upon healthy persons and upon rabbits, and found a great reduction in the hemoglobin after ether anesthesia. Leukocytosis was also pronounced in many of these experiments, and the general course of the blood returning to the normal after anesthesia was found to be the same in man as in animals. It required from twenty-four to forty-eight hours for the restoration of the blood to normal.†

Drugs.—A variable degree of leukocytosis may follow the administration of drugs by mouth, subcutaneously, or by the rectum. By the first method, ether, chloroform, tincture of gentian, oil of peppermint, oil of anise-seed, camphor, thyroid extract, bicarbonate of soda, caffein, quinin, bismuth, and after purgation with castor oil, scammony, and podophyllin, the leukocytes are increased. Cabot* reports a series of instances where leukocytosis has followed toxic doses of opium, chloral, caustic potash, corrosive sublimate, belladonna, nitric acid, carbolic acid, arsenic, and alcohol.

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* "Annals of Surgery," 1901.

† Anders and Boston, "Proc. Phila. Col. Physicians," June, 1904.

A similar phenomenon is observed after the intravenous administration of uric acid, pilocarpin, antipyrin, urate of soda, nuclein, nucleic acid, hemi-albumose, peptone, pepsin, tuberculin, pyocyanin, extract of bone-marrow, thymus gland, and spleen, all of which are followed by a slight temporary reduction in the peripheral leukocytes, and later by leukocytosis.

The degree of leukocytosis is often parallel to that of the local irritation produced, as is exemplified by the leukocytosis following the subcutaneous injection of weak acids or alkalis; and from the local application of mercury, antimony, croton oil, etc. Large doses of either bacteria or their toxins reduce the number of leukocytes; while moderate doses give no constant finding, since the individual animal resistance is capable of permitting or preventing wide fluctuations; but commonly a diminution in the leukocytes ensues, and is a precursor of leukocytosis.

Leukopenia (hypoleukocytosis) is a condition when the number of leukocytes found in the peripheral circulation is below that of the normal for a given individual. Leukopenia may result from malnutrition, prolonged cold baths, hot baths; infectious conditions wherein there is no leukocytosis may present these phenomena; *e. g.*, measles (early stage), rötheln, tubercular meningitis, la grippe, purely tuberculous conditions, typhoid fever (late in its course), and malaria. When the result of starvation, and in typhoid fever, the number of lymphocytes is relatively increased. Leukemia when complicated by an infection displays this phenomenon. Pernicious anemia, splenic anemia, Hodgkin's disease, the anemia of syphilis, "Dum-dum" fever (tropical anemia with splenic enlargement), and hemorrhage may display leukopenia.

Lymphocytosis.—Lymphocytosis consists of both an absolute and a relative increase in the number of circulating lymphocytes. This increase must be compared with the normal number of lymphocytes for each individual in order to establish the presence of lymphocytosis. Again, lymphocytosis may co-exist with an increase of the total number of leukocytes (lymphatic leukemia).

The number of lymphocytes is high in the blood of healthy infants—50 to 60 per cent.—during the first few weeks of extra-uterine life; and is also apt to be increased in many of the diseases of childhood; while in adults there are normally 20 to 30 per cent. of small and 4 to 8 per cent. of large lymphocytes. The increase may affect principally either the smaller or the larger lymphocytes, and it is not uncommon to find these cells quite uniform, rendering it impossible to classify them according to size (Plate 9).

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*"Clin. Exam. of the Blood," fifth ed., pp. 415-419.

Caution.—Be slow to diagnose lymphatic leukemia in the young, since the blood of rickets, hereditary syphilis, scurvy, variola, diarrhea, gastro-intestinal disorders, malnutrition, and pertussis often shows an increase in the number of lymphocytes. During convalescence from exfoliating dermatitis, pertussis, and diarrhea, I have seen the number of lymphocytes equal 55 to 65 per cent. of the total number of leukocytes.

Clinical Significance.—In children the degree of lymphocytosis serves as a fair index to the child's development in cases where it is possible to exclude other etiologic factors. It is also of limited value in diagnosing whooping-cough before the characteristic symptom appears. The large lymphocytes are increased in certain splenic tumors, and in measles after the temperature has reached the normal.

False Lymphocytosis.—The blood of adults not infrequently shows false lymphocytosis, a condition wherein the apparent increase in the number of lymphocytes results from the absolute diminution of the polymorphonuclear cells; and is observed in general debility, pernicious anemia, chlorosis, Graves' disease, syphilis, cervical adenitis, hemophilia, goiter, and typhoid fever.

Absolute Lymphocytosis.—Absolute lymphocytosis is the term used to denote a condition wherein the total number of leukocytes is increased, and there is an abnormally high percentage of lymphocytes present. It is characteristic of lymphatic leukemia; and has been observed by Cabot in two cases of bronchopneumonia complicating whooping-cough.

In a case of exfoliating dermatitis (boy of eight years) studied at the Philadelphia Hospital in 1897, I found the leukocytes to number 55,000 to 65,000 per cubic millimeter, 60 per cent. of which were lymphocytes. Three months later the leukocytes were practically normal.

Absolute lymphocytosis occurring in conjunction with glandular enlargement constitutes the diagnostic feature of lymphatic leukemia. Cabot regards lymphocytosis with eosinophilia as suggestive of obscure syphilitic disease. Lymphocytosis may be excited by thyroid extract, pilocarpin, and tuberculin.

Eosinophilia.—A condition of the blood characterized by an increase in the number of eosinophils (see Plates 5–7 and 8, eosinophils and eosinophilic myelocytes).

Physiologic eosinophilia is observed during infancy and menstruation.

Pathologic eosinophilia may be found in the following conditions: Fibroid bronchitis; bronchial asthma, and when dyspnea is a prominent feature where they have been known to reach 50 per cent. of the total number of leukocytes (see *Sputum*, page 445); and in certain acute and chronic cutaneous maladies, as urticaria, where I have seen them equal 62 per cent.; prurigo and psoriasis, where they were noted to reach 17 per cent. by Cannon, and 22 per cent. in a case observed personally at the dermatologic clinic of the Medico-Chirurgical Hospital. I have also observed at the same clinic eosinophilia in connection with herpes zoster, pemphigus, dermatitis, ichthyosis, and chronic eczema. In gonorrheal urethritis increasing eosinophilia suggests extension of the trouble to the posterior urethra. In prostatitis the eosinophils are often increased.

Parasitic Eosinophilia.—Trichinosis, filariasis, and infection with Bilharzia hæmatobia* may show a variable degree of eosinophilia early, but later eosinophilia becomes less marked, and may even be absent, as has been the course of a case of trichinosis which I have been privileged to follow for a period of five years. Calvert† studied two cases of filariasis, in one of which the blood showed from 12.33 to 22 per cent. of eosinophils; while the second presented from 6 to 20 per cent. Leukocytosis existed in both instances, varying from 26,666 to 14,000 per cubic millimeter.

Bückler concludes from a series of investigations that all varieties of helminthides are capable of causing an increase in the number of eosinophils in the blood. He found with infection by the oxyuris 16 per cent. of eosinophils; ascarides, 19 per cent.; ankylostoma, 72 per cent.; and Tænia mediocanellata, 34 per cent. (Ehrlich and Lazarus, p. 429; see also *Ankylostoma*, page 143).

Malignant Tumors.—Eosinophilia may be found in connection with malignant growths, sarcoma commonly ranging from 8 to 12 per cent.; but in lymphosarcoma the percentage may be greater.

Eosinophilia may follow the removal of the spleen or when this organ fails to functionate. Hemorrhagic exudates and purpura hæmorrhagica are often accompanied by a high-grade eosinophilia. In a case of chylous ascites (tubercular) I have found 38 per cent. of eosinophils in the peripheral circulation. Klein found 74 per cent. of eosinophils in the hemorrhagic pleural effusions, with an increase in the number found in the peripheral circulation. Eosinophilia is a common feature of myelogenic leukemia (see *Leukemia*, page 130). In leprosy I found 8.7 per cent. of eosinophils.*

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* "Lancet." Dec. 6, 1902, p. 1540.

† "Johns Hopkins Hosp. Bul.," Jan., 1902, p. 23.

Medicinal eosinophilia may follow phosphorus-poisoning and, according to Taylor, the administration of nuclein and pilocarpin. The eosinophils are increased to 9 per cent. by the administration of camphor. During the height of the course of infectious fevers the eosinophils are found to be diminished, and at times absent—scarlet fever and rheumatic fever serving as exceptions to this rule. They are also diminished by the administration of ether as an anesthetic (Anders and Boston).

During the post-febrile period it is common to find an increase in the number of eosinophils, which ranges from 9 to 90 per cent. of the total number of leukocytes.

Clinical Significance.—Eosinophilia is of great service in diagnosing trichinosis, filariasis, tumors connected with the genital tract, and has been found by me in leprosy. In malignancy it is usually conceded to follow the existence of metastasis, and when co-existent with lymphocytosis, favors obscure syphilis. An increase in the eosinophils accompanies the uric-acid diathesis. Eosinophilia may be regarded of favorable prognostic value when it develops during the course of scarlet fever, pernicious anemia, chlorosis, and after hemorrhage. In obscure tumors of the viscera eosinophilia tends to exclude malignancy.

Diminution of the Eosinophils (Hypoeosinophilia).—The eosinophils are diminished or absent during the height of acute fevers, as typhoid, diphtheria, pneumonia, la grippe, and the majority of febrile conditions which display leukocytosis during some part of their course, during digestion, and after vigorous muscular exercise. This diminution in the number of eosinophils does not depend upon high fever, since an increase may be observed in scarlet fever, rheumatic fever, and malaria. Hemorrhage and malignancy are often associated with hypoeosinophilia, and it is said to follow castration (Neusser).

BACTERIOLOGY OF THE BLOOD.

There are valid reasons for the belief that initially most blood infections are local; that after a lowering of the normally present protecting power, the barriers are broken and secondary infection ensues (*bacteremia*). Such invasion results in streptococcemia, staphylococcemia, bacillemia, or diplococcemia. Among the bacteria that are to be found free in the circulation are the following: Streptococcus, bacillus of anthrax, Bacillus enteritidis, Bacillus pyocyaneus, Bacillus proteus vulgaris, bacillus of glanders, bacillus of Friedländer, bacillus of tuberculosis, typhoid bacillus, paracolon bacillus, colon bacillus, bacillus of Sternberg, Bacillus pestis, Bacillus lepræ, vibrio of cholera, spirillum of relapsing fever; and the Micrococcus melitensis, gonococcus, pneumococcus, Diplococcus intracellularis, Micrococcus tetragenus, diplococcus of scarlatina (Class); and micro-organisms have been found in the blood in such diseases as chorea, purpura hæmorrhagica, rheumatism, scurvy, sepsis, mumps (diplobacilli), etc. One observer reports having found a diplococcus in the blood of typhus fever. I was unable to confirm this report in the study of two cases at the Philadelphia Hospital.

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* "Trans. Phila. Co. Med. Soc.," Jan., 1903.

THE REACTION OF BACTERIA AND THEIR PRODUCTS OF THE BLOOD.

Many of the changes that appear in the blood, commonly regarded as being the effect of fever, are in reality caused by the action of bacteria or their products; and such bacteria may be the direct cause of the disease from which the individual is suffering. The researches of Bouchard, Gley, and others have shown that in the bodies of certain organisms (Bacillus pyocyaneus) and in their filtered products were substances capable of producing a vasoconstricting action. This serves to explain the polycythemia present in certain febrile conditions (see *Polycythemia*, page 84). The injection experimentally of peptone, animal extracts, tuberculin, and pyocyanin produces in a similar manner concentration of the blood. Grawitz* has detected decided variation in the density of the blood after the injection of pure cultures of diphtheria, the various pus-producing organisms, and the vibrio of cholera. The isotonic tension of the blood was found to be increased by small doses of toxins, while large doses, and even the injection of typhoid bacilli, produced an opposite effect.† The various products resulting from the development of bacteria in the human organism—ptomains, toxalbumins, and bacterioproteins—are generally conceded to be the active and destructive agents of the albumins of both the blood and body tissues in all infectious conditions. Changes in the body tissues are not of necessity associated with fever. The chemotactic action of both bacteria and their products upon the leukocytes is displayed through the production of leukocytosis (see *Leukocytosis*, page 101).

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* "Zeit. f. klin. Med.," vol. xxii, p. 411.

† Bianchi and Mariotti, "Wien. med. Presse," 1894, No. 36.

METHOD OF COLLECTING BLOOD.

1. Sterilize the skin of the flexor surface of the arm above and below the elbow, when an assistant should grasp the arm so as to prevent the venous return of the blood and thereby distend the superficial veins. My own practice is to make an incision through the skin and allow the assistant to draw its edges aside by the aid of two tenacula, when it is possible to enter the vein directly and thus avoid possible contamination by passing the needle through the skin.

2. Into the most prominent vein plunge a hypodermic needle. When the needle enters the vein, the blood usually begins to flow into the bulb of the syringe, and this should be gradually enhanced by withdrawing the piston slowly until about one-half cubic centimeter of the blood has been removed. Withdraw the needle quickly and apply a surgical dressing to the site of the wound.

3. Immediately expel the blood contained in the syringe into tubes containing agar-agar which is heated to a temperature of 42° C. (just sufficient to liquefy the medium and not to interfere with bacterial growth). After the addition of one or more drops of blood to the tube, shake well, and stand in a cool place until the medium is solidified. It is well to incline the tubes slightly in order to obtain surface growths. Tubes or flasks (Fig. 42) containing bouillon may be inoculated with the blood in this manner, and it will be found a most convenient method of inoculating tubes for plating.

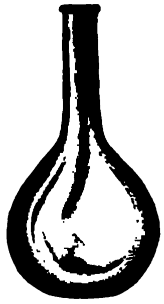

Fig. 42.—Koch flask.

Personal experience favors the plan of mixing the blood with a liquid culture-medium and then inoculating other tubes with this mixture. The only objection which can be raised to the diluting of the blood before it is used for inoculation of tubes is the liability of contamination, but this danger, with our present bacteriologic technic, is reduced to a minimum.

Caution.—It is of special moment that only a few drops of blood (1 to 10) be introduced into a tube containing 10 c.c. of culture-medium, since the blood often contains certain properties the result of bacterial development (agglutinins and bacterial products), which, unless highly diluted (1-50 to 1-250), interfere with the growth of the bacteria, and at times prove germicidal to them. Cultures from the blood should be kept at an incubating temperature (37.5° C.) for at least twenty-four hours, and in the case of the tubercle bacillus, for from ten days to two weeks—glycerin-agar being employed as a culture-medium.

Collecting blood in this manner for bacteriologic study does not cause more pain than does the introduction of the hypodermic needle, and if done under thorough aseptic measures should not be attended by ill results. The gonococcus may be cultivated in low dilution (1-2) on a medium of agar and blood-serum; and special media will also be necessary in the cultivation of the bacillus of influenza and the Bacillus aërogenes capsulatus, for which the reader is referred to special works on bacteriology.

Blood from the peripheral circulation when smeared upon slides or cover-glasses may show bacteria, but this finding is rather unusual and a negative result would be of no clinical moment; while a positive result, which is seldom obtained, bears some clinical significance. It is possible to obtain the bacillus of leprosy in this manner by making a puncture into the skin overlying one of the tubercles; and I have obtained smears showing the bacillus of leprosy within the bodies of the leukocytes, in blood drawn through the healthy skin (see *Leprosy*, page 112). Blood collected from near the initial wound, in tetanus, often contains the tetanus bacillus. The bacillus of anthrax is commonly found in this manner and, with less frequency, the Bacillus mallei.

Films.—Smear the blood upon slides, exerting but little pressure, since a spread far too thick for ordinary study is preferable. Immerse the dried films in a 5 per cent. aqueous solution of acetic acid for ten seconds to remove the hemoglobin from the erythrocytes; dry by blowing upon the specimen, and then hold it, face down, over the open mouth of a bottle containing ammonia to neutralize the remaining acid.

Staining.—Staining may be accomplished by the ordinary anilin dyes (carbolfuchsin, anilin-gentian-violet, etc.). Gunther recommended staining with the latter solution for twenty-four hours, then treat with 1-14 solution of nitric acid until a light-green color develops; wash in alcohol, dry in the air, and mount. The erythrocytes do not stain after treatment with acetic acid, and bacteria, when present, comprise a conspicuous feature of the specimen.

Animal Inoculation.—It has been my practice to always have at hand a healthy guinea-pig for the purpose of inoculating with the blood obtained for bacteriologic study, and to inject from five to twenty minims either beneath the skin or into the peritoneal cavity of the animal. Certain hemic changes follow similar injections with normal blood—loss of hemoglobin and leukocytosis; but bacteremia, abscess, or death of the animal results from the introduction of bacteria.

BACTERIA OF THE BLOOD.

Anthrax.—The bacillus of anthrax was first detected in the blood by Pollender in 1849. The bacilli are best demonstrated in the blood by inoculating an animal with a virulent culture and studying its blood within twenty-four hours. They are always present in the heart's blood at postmortem, may be transmitted from the mother to the fetus, and have been found in the liquor amnii.

Relapsing Fever.—Obermeier in 1873 described a flexible spiral organism, about 0.1 μ in diameter and from 20 to 40 μ in length, as occurring in the blood of persons suffering from relapsing fever during the paroxysmal stage of the disease (Fig. 43). Since the original report of Obermeier little further has been learned regarding this organism. During the paroxysm they are generally very numerous, occurring as long, slender, and flexible spirochæta, and display vigorous movements. Their extremities are pointed. The spirillum of Obermeier stains readily by the ordinary dyes (two to five minutes), but not by Gram's method. Efforts to cultivate the organism have been unsuccessful; Koch, however, is reported to have cultivated the spirillum, and to have demonstrated the formation of spores. During the interval between paroxysms there are to be seen small, refractile globules (coccus like), which v. Jaksch believes transform into spirochæta. After the crisis they are found to be motionless, and many of them have been engulfed by the leukocytes.*

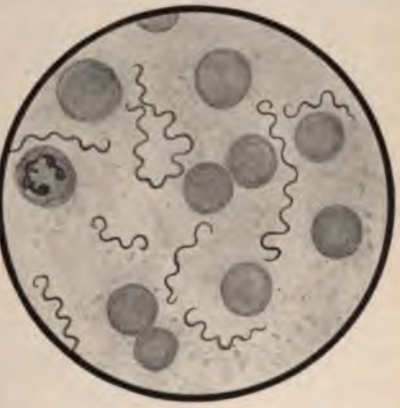

Fig. 43.—Spirilla of relapsing fever in human blood (× 1000).

Albrecht has described at length an interesting series of observations.† He collected the blood, during the afebrile period, in moist chambers, and after from five to six days many of these specimens became loaded with spirilla. The spirillum may be kept living for a long time—"one hundred and eighty days"—in defibrinated blood at a temperature of 20° C. It is destroyed by fluid from the serous sacs, urine, saliva, oxygen, and CO_2. The disease may be transmitted to both man and apes by inoculation with the blood of infected patients.

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* "Annal. de l'Institut Pasteur," 1891, p. 545.

† "Deut. Archiv f. klin. Med.," vol. xxix, p. 77.

Tubercle Bacillus.—The tubercle bacillus is to be encountered in the blood of acute miliary tuberculosis, and at the same time the bacilli may also be found in the urine without the existence of tuberculous lesions of the genito-urinary tract. Tubercle bacilli have also been found in the blood of the fetus when born of a tuberculous mother. It does not follow, however, that tubercle bacilli are at all a common finding in the blood. The various observers have found the blood of tuberculous persons to contain staphylococci and streptococci; while negative results have been equally numerous. The method most likely to prove satisfactory is the inoculation of a guinea-pig with blood from a person known to be tuberculous.

Leprosy.—The Bacillus lepræ may be found in the blood during the advanced stage of this disease, yet, generally speaking, it rarely enters the circulation. When an incision is made directly over a tubercle and pressure made upon this mass, the blood which exudes is likely to contain lepra bacilli. Streker* found lepra bacilli in the leukocytes in all of five cases examined; while Brown† detected these bacilli in the polymorphonuclear leukocytes in nine out of sixteen cases examined. In a case studied through the courtesy of Dr. John V. Shoemaker blood drawn from a rather deep incision through the normal skin of the finger was prepared in the usual manner, fixed by heat, and stained for the "Bacillus lepræ" with carbolfuchsin, and counterstained by Gabbett's methylene-blue solution. Such specimen smears contained few polynuclear leukocytes, in the protoplasm of which lepra bacilli were readily seen; the smallest number of bacilli observed in a polynuclear leukocyte being two, and the greatest number eight. Bacilli were also found free in the plasma, and in this situation occurring singly or at most two or three in a single field (Plate 5).‡ Streker found extracellular bacilli in all of his cases.

Typhoid Fever.—Bacillemia is not an uncommon concomitant of typhoid fever, though until recently typhoid bacilli were

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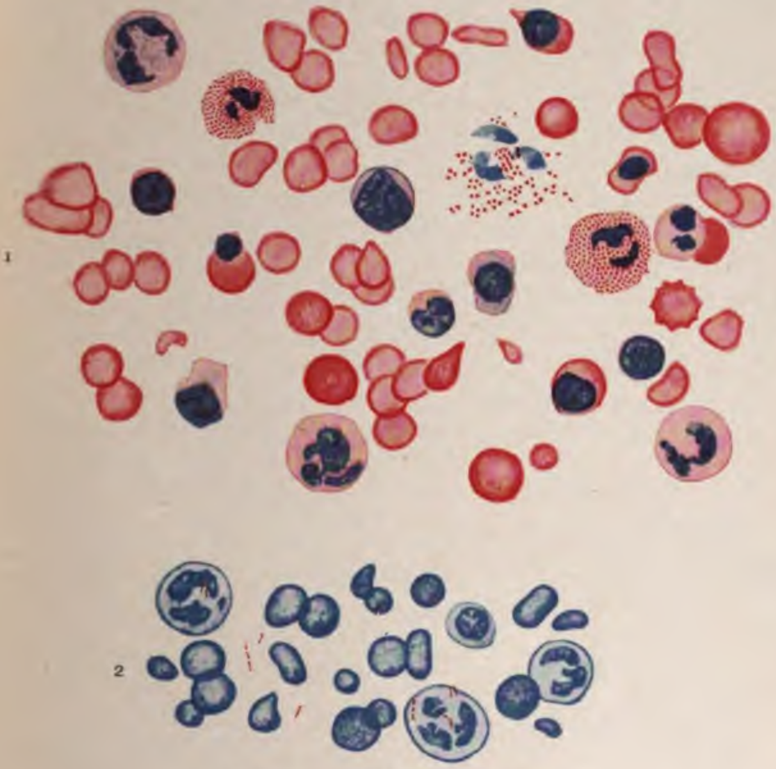
* Münchener med. Wochenschr., [illegible]

† Trans. Pa. Med. Soc., [illegible]

‡ Proc. Phila. Co. Med. Soc., Jan., [illegible]

PLATE 5.



THE BLOOD-CELLS IN LEPROSY.

1. Blood from a case of leprosy stained with eosin and hematoxylin. Note the numerous nucleated erythrocytes; at the right, one showing two nuclei; at the left, one with nucleus situated on the margin of the cell.

2. Blood stained with carbolfuchsin and methylene-blue, and showing lepra bacilli (obj. Queen one-twelfth oil-immersion).

not supposed to enter the blood. In mothers suffering from typhoid the bacillus of Eberth has been repeatedly isolated from the blood of both the fetus and the placenta. R. C. Rosenberger, in an exhaustive monograph,* to which is appended a complete bibliography, collected from the literature reports of 518 cases of typhoid fever wherein the blood was examined for the bacillus of Eberth, with positive results in 419 of the cases, or 80.8 per cent. Typhoid bacilli may be found in the blood early during the course of the disease. The bacillemia has not been shown to bear a direct relation to the occurrence of the bacillus of Eberth in the urine, yet were the blood and urine to be studied correlatively, it is highly probable that a more or less direct relation would be found to exist between bacillemia and bacilluria.

Suppuration.—The ordinary pus-producing organisms, the streptococcus, staphylococcus, and Bacillus pyocyaneus, have been isolated from the blood in cases wherein suppuration existed and in chronic tuberculosis. Von Eiselberg† found specific bacteria in the blood of 77 out of 156 cases of sepsis examined. Among the organisms found were the streptococcus, staphylococcus, pneumococcus, gonococcus, and the Bacillus coli communis. He also isolated specific bacteria from the blood in 1 out of 3 cases of puerperal sepsis. The Bacillus enteritidis, streptococcus, staphylococcus, and Bacillus coli communis have been found in the placental blood of women suffering from, apparently, local sepsis. An infant born of a mother suffering from facial erysipelas showed streptococcemia and ulcerative endocarditis. Blood withdrawn from the vein during the course of erysipelas is likely to be free from bacteria, but taken from the vicinity of the infected area it is apt to contain cocci, as has been repeatedly demonstrated by the author.

Pneumonia.—During the course of croupous pneumonia the pneumococcus may be found in the circulating blood and in the fluid from infected joints. Rosenow‡ obtained cultures of the pneumococcus in 160 out of 175 bloods examined; positive results were obtained in all stages of the disease and in 47 out of 80 cases studied by blood smears. In reports of 56 examinations collected from the literature (Rosenberger) diplococci were found in 29 instances. Beco isolated the bacillus of Friedländer from a case of pneumonia due to this organism. I have isolated the bacillus of Friedländer from the heart's blood, meninges, cardiac abscess, and from the lung in a subject dead of pneumonia.

Influenza.—Cannon recovered short bacilli from the blood in 20 cases of influenza, but this organism has also been obtained from the blood of scarlet fever, measles, chicken-pox, and diphtheria, which renders this finding of limited value.

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* "The Bacteriology of the Blood," "Amer. Jour. Med. Sci.," August, 1903.
† "Wien. klin. Wochenschr.," 1890.
‡ "Jour. Amer. Med. Assoc.," March 18, 1905, p. 871.

Epidemic Meningitis.—In epidemic cerebrospinal meningitis Gwyn,[*] among other observers, has recovered the Diplococcus intracellularis of Weichselbaum (Fig. 213) from the venous blood during life. I have also obtained this organism from the heart's blood at postmortem, but efforts at its cultivation before the patient's death have been, with but a single exception, of no avail. In one instance I recovered a pure culture of the diplococcus of pneumonia from the venous blood antemortem, and from the cerebrospinal fluid both the pneumococcus and the Diplococcus intracellularis were cultivated during life and at postmortem. In another case a bacillus (colon group) was cultivated from the venous blood antemortem, and the colon bacillus and the Diplococcus intracellularis were recovered from the meninges at autopsy. The pneumococcus has been isolated from the blood of the fetus where the mother suffered from cerebrospinal meningitis.[†]

Purpura.—Letcherich found large bacilli in the blood of purpura hæmorrhagica, while other observers have recovered cocci. In three cases studied antemortem I was unable to cultivate bacteria from the venous blood.

Ulcerative Endocarditis.—In acute ulcerative endocarditis the various pus-producing organisms have been cultivated from the blood, and in addition to these Gwyn[‡] has recovered the Bacillus aërogenes capsulatus.

Gonococcus.—Johnson,[§] among other observers, has cultivated the gonococcus from the blood of persons suffering from gonorrheal endocarditis.

Plague.—In bubonic plague bacillemia occurs, as is shown by Atkinson's[‖] study of 273 cases, in 221 of which the Bacillus pestis was isolated from the blood (81 per cent.). Rosenberger has collected reports of blood examinations of 433 cases of bubonic plague with positive results in 300 instances (69 per cent.).

Diphtheria.—The diphtheria bacillus may be cultivated from the blood in malignant forms of the disease.

Fungi.—In connection with general furunculosis Weber[**] recovered blastomycetes from the blood.

Malta Fever.—During the course of Malta fever the Micrococcus melitensis may be recovered from the blood of the venous circulation, and when cultivated from this source will be found to agglutinate with the patient's serum (see page 125).

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[*] "Johns Hopkins Hosp. Bul.," 1899, p. 112.

[†] "Schmidt's Jahrbuch," 1893, p. 227.

[‡] "Johns Hopkins Hosp. Bul.," 1900, p. 185.

[§] *Ibid.*, Oct., 1902.

[‖] "Lancet," Jan. 26, 1901.

[**] "Society of Internal Med.," Berlin, Dec., 1902.

Chorea.—Leredde obtained a pure culture of the Staphylococcus pyogenes albus from the blood of a case of chorea complicated by endocarditis. In chorea the cadaveric blood has been found to contain bacteria.

Scarlet Fever.—During the course of scarlet fever Raskin* found a Staphylococcus pyogenes in the circulating blood in 2 out of 64 cases examined. Class has found the diplococcus of scarlatina to invade the circulation; while other observers have conducted systematic researches which were attended with negative results.

Scurvy.—According to Rosenell, Murri, Muller, and Wieruszky bacteremia may occur during the course of scurvy, but the latter of these observers was unable to show any relation between the bacteria recovered from the blood of scorbutic patients and this disease. In elephantiasis, valvular heart disease, and carcinoma bacteria have been isolated from the blood.

Leukemia.—The literature contains detailed bacteriologic reports of 96 cases of leukemia, 49 of which were chronic.† In nearly one-third of the cases bacteriologic study was negative, and many bacteria were classified only as cocci or bacilli. Among the determined organisms were Streptococcus pyogenes, Staphylococcus citreus, aureus, and albus, pneumococcus, sarcinæ, bacillus of Friedländer, Bacillus proteus, and the colon bacillus. In 12 of the cases short, thick bacilli with rounded ends were found; and spore-formation was suspected or noted occasionally, while clear central spaces in the bacilli were repeatedly mentioned.

INVASION OF THE RED BONE-MARROW BY BACTERIA.

E. Fraenkel‡ reports a series of observations upon the red bone-marrow during the course of the various acute infectious maladies. It does not always follow, from these observations, that the specific organism of the disease from which the patient is suffering is the one found to invade the bone-marrow. Secondary infection of the bone-marrow during the course of acute febrile conditions was found to be rather common.

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* "Centralbl. f. Bakt.," 1889, p. 286.

† Nichols, "Amer. Med.," July 18, 1903, p. 102.

‡ "Mitt. a. d. Grenzgeb. d. Med. und Chirurg.," vol. xii, p. 419.

Typhoid Fever.—Fraenkel recovered typhoid bacilli from the bone-marrow of patients suffering from typhoid fever during all stages of the disease, and makes the assertion that typhoid bacilli are invariably present in the bone-marrow. In this connection the reader is referred to the invasion of the blood by typhoid bacilli, page 112, since the question of bacillemia must of necessity cause an invasion of the bone-marrow with typhoid bacilli. Typhoid bacilli remain in the bone-marrow after the local lesions in the intestine are healed.

Tuberculosis.—In chronic pulmonary tuberculosis specific bacteria are rarely to be recovered from the bone-marrow; nor is it at all common to find the organisms of secondary infection present in the marrow in this malady.

Diphtheria.—It is extremely unusual to recover the diphtheria bacillus from the bone-marrow during the course of diphtheria, a condition readily explained through the fact that bacillemia is not a common feature of diphtheria. Fraenkel found the bone-marrow to be invaded with streptococci in many of the cases of diphtheria.

Scarlet Fever.—In scarlet fever streptococci are an almost constant finding in the bone-marrow. There appears to be no constant period, during the course of the disease, at which time the streptococci enter the bone-marrow; and the period during which they may be recovered from this situation varies greatly with different types of the disease.

Erysipelas.—In erysipelas specific bacteria may be found in the bone-marrow during the acute stage of the disease. These organisms are to be found in large numbers after the local symptoms have subsided.

Clinical Significance.—The direct injury that the bacteria must exert upon the bone-marrow serves to explain, in a measure at least, the blood changes common to this and other infectious maladies. Infection of the bone-marrow with bacteria appeals to one as the possible foundation for many, and possibly all, the hematologic changes present in acute infectious conditions.

The recovery of specific bacteria from the red bone-marrow does not appear to be a clinical finding worthy of special description, since the same bacterium may be more easily cultivated from the blood.

SERUM-DIAGNOSIS.

Serum-diagnosis is based upon a study of the pathologic chemistry of the blood, and has been found of the utmost importance in the recognition of disease, though it is only possible at present to detect the presence of a limited number of varieties of infection. The reactions that take place through serum-diagnosis may be conveniently classed under the following headings:

I. The reaction between a pure culture of a micro-organism and the serum of an animal infected with the same organism: (*a*) The agglutination; (*b*) solution of the micro-organism; (*c*) formation of a tangled mass composed of motionless filaments of micro-organisms; (*d*) the production of a precipitate when serum of an infected animal and the filtered culture (germ-free) of the bacterium causing the infection are mixed.

II. Reaction between erythrocytes of one species of animal and the serum of another species into which the erythrocytes of the former animal have been injected: (*a*) Agglutination of the erythrocytes; (*b*) solution of the erythrocytes with the formation of a precipitate should the serum of the animal injected be mixed with that of an animal of the same species as the one from which the blood-corpuscles were taken. Neisser and Daring have shown that the usual reaction between human blood-serum and the blood of animals of another species is curiously modified in uremia. Human serum (active) $\frac{1}{10}$ c.c., dissolves the blood-cells in 1 c.c. of rabbit's blood, and the fluid becomes clear in two hours. After the thermolabile complement of the serum is destroyed by heat and a quantity of it mixed with the native serum, the addition of the rabbit's blood will not affect the usual reaction, but the fluid remains cloudy and the corpuscles are not dissolved.

Streptocolysis.—It has been shown by various observers, and more recently by G. F. Ruediger,* that virulent streptococci when grown in heated rabbit's serum and other serums develop a hemolysin which is capable of destroying the erythrocytes of several animals. This product (hemolysin) is an organic substance which is destroyed by a temperature of 70° C. for two hours; deteriorates at incubating and room temperatures; but may be kept for a longer period in a refrigerator; is non-dialyzable, and is destroyed by peptic digestion.

Streptocolysin is composed of a haptophore and a toxiphore group which appear to be rather firmly united. Chickens' serums are capable of neutralizing the haptophore group; while the toxiphore group is destroyed by zinc chlorid. The red cells of man and of the rabbit are least resistant. The cells of the chicken, ox, and horse are hastened to dissolution by first washing them in normal salt solution.

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* "Jour. Amer. Med. Ass.," Oct. 17, 1903.

The serums of certain animals have an ***antistreptocolysin.*** Formaldehyd in weak solution exercises an antihemolytic power. The filtered culture of a virulent streptococcus in heated serum will be found toxic for rabbits.

III. Reaction between normal blood-serum and bacteria, which consists in both agglutination and solution.

IV. Reaction between a pure culture of one class of bacteria and the erythrocytes.

V. Reactions between both normal and pathologic serums of animals and the blood-corpuscles of animals of the same or of other species, which consist of solution and agglutination.

VI. A reaction occurs between the serum of one species of animal and an emulsion of cells derived from the organs of another species, the animal having been previously injected with the emulsion, and consists in solution, except when the cells are motile (spermatozoa), when agglutination occurs. There is a reaction which also takes place within the body after active serums derived from inoculated animals or from normal animals of certain species are injected experimentally. This change is termed hemolysis, and simulates that found in pernicious anemia.

VII. Halban and Landsteiner * have recently shown that the serum of maternal blood is far more active than is that of fetal blood—viz., (*a*) It requires a larger number of corpuscles to undergo solution; (*b*) agglutinates blood-corpuscles more actively; (*c*) has a stronger action against bacteria and against the process of fermentation; and (*d*) is more potent as an antitoxin and against immunizing serum. This explains the readiness with which the new-born are infected. A point of practical importance is to ascertain at what period of extra-uterine life infantile serums become active. The specific agglutinin results in the precipitation of all bodies suspended in the media. Non-specific agglutinin is associated with a precipitate of the motile bodies only, the bacteria remaining passive. The hanging-drop method may be applied in the study of hemoprecipitins.†

WIDAL REACTION.

Most characteristic and valuable among the reactions for serum-diagnosis is that of the Widal reaction, which is usually referred to in connection with typhoid fever, but the same technic employed in this reaction, with slight deviation, serves as a basis for the various other serum reactions.

Method.—Collecting of the Blood.—Two methods are in vogue for collecting the blood for the Widal reaction: (*a*) The dry method, in which a piece of glazed paper is allowed to touch the summit of a drop of blood, and is dried in the air; (*b*) by collecting the blood in the capillary pipet and diluting. Wright has suggested a special pipet for collecting the blood (Fig. 44), and Widal has found that even a less complicated pipet serves well for this purpose. (1) Draw the blood into the pipet; heat the extremities to seal them off, after which the pipet and its contents are conveyed to the laboratory. (2) After sufficient time has elapsed for the serum to separate from the clot, break off the ends of the pipet and expel the serum into a sterile watch-glass (labeled). (3) By means of a graduated pipet, transfer a portion of the serum to another watch-glass containing saline solution. In this manner dilutions 1–20, 1–100, 1–500 or more are readily accomplished. Wright's tubes are easily made from the glass tubing always at hand in the laboratory.

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* Anatomical Institute, Vienna.

† Robin, "Phila. Med. Jour.," Dec. 20, 1902, p. 1019.

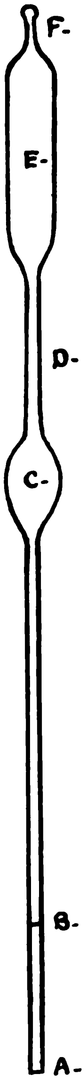

Fig. 44.—Wright's pipet (actual size): A, Open end of capillary tube; B, index; C, mixing chamber; D, connecting capillary tube; E, air-chamber; F, sealed end.

Culture.—A pure culture of typhoid bacilli is transferred to a series of agar slants, making one or more cultures each day. Place in the dark at room temperature, and when the culture is from ten to fourteen days old, transfer it to bouillon and allow the second culture (bouillon) to remain at room temperature for twenty-four hours. By this method the typhoid bacilli develop to a large size, and their movements are rather sluggish when studied correlatively with bacilli grown at incubating temperature (37° C.), and spontaneous clumping is rarely seen. I have employed this method during the past six years and have had no reason to modify it in any way. It is the method now in use by the Board of Health of Philadelphia, and its perfection is due to the careful work of Dr. Stewart, Director of that department.

I. When the blood has been collected upon glazed paper, add 20 to 40 times its quantity of sterile bouillon to the drop, when make gentle friction with a platinum loop over the surface of the drop at its margin, thereby assisting in its solution and the mixing of the serum with the bouillon. In this way an approximate dilution of 1–20 or greater is obtained.

II. Place a drop of the mixture (blood and bouillon) upon the center of a cover-glass, and to it add a small drop of the twenty-four-hour bouillon culture of typhoid bacilli; mix gently.

III. Place a ring of vaselin just beyond the margin of the excavation on a hanging-drop slide (Fig. 45), and then invert the slide over the cover-glass, taking care that the drop of liquid upon the cover-glass corresponds to the center of the excavation upon the slide. Press the slide firmly upon the cover-glass to which it adheres. Invert the slide quickly and the specimen is now ready for examination under the microscope.

Caution.—Should too much vaselin be employed or the slide be turned slowly or should the drop of liquid be too large, the fluid will collect around the margin of the excavation. Properly prepared, the drop occupies the center of the cover-glass suspended from its under surface into the chamber of the slide. When the blood has been collected in the pipet, break the extremities of the tube and force out 4 or 5 drops of the serum by blowing; then place a small drop upon the center of the cover-glass, add salt solution or bouillon to effect the dilution, and follow by adding a drop of the typhoid culture, and proceed as previously outlined. Place the specimen upon the microscope under a one-fifth or, better, a one-sixth objective, and adjust the mirror, condenser, and iris diaphragm so as to permit of a moderate amount of light.

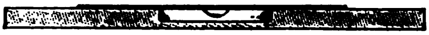

Fig. 45. — Lateral view of a hanging-drop slide, cover-glass in position.

To reiterate, a one-sixth objective focuses at a point just sufficiently distant above the cover-glass to enable one to see between the cover-glass and the tip of the lens when the eye is brought on a plane with the table of the microscope. A common source of annoyance is the breaking of the cover-glass by the objective. Place the eye in position and elevate the objective gradually, when the motile bacteria come into view. Should there be no evidence of a reaction, actively motile bacilli are seen equally disseminated throughout the field.

The Reaction.—The various steps in the reaction may be classified as follows: (*a*) Place the center of the drop of liquid under the objective, and actively motile bacilli will be seen throughout the entire field. (*b*) The first evidence of a reaction is detected by retarding movements of the bacilli. (*c*) A small bacillus attaches itself to the side of a larger organism,—the Y formation,—and later other bacilli are seen to come in contact with these; some of them remaining to form a clump, while others by active movements detach themselves from the clump and again appear free in the liquid. As the movements of the bacilli lessen, more and more bacilli become attached (Y formation), until there are decided aggregations of bacilli in certain areas throughout the entire drop of liquid (Fig. 46). (*d*) The reaction is not complete until all motility of the organisms composing these various aggregations has ceased.

Caution.—Clumping may be found at the margin of the drop when the test is made with normal blood; therefore only central clumping is of clinical value.

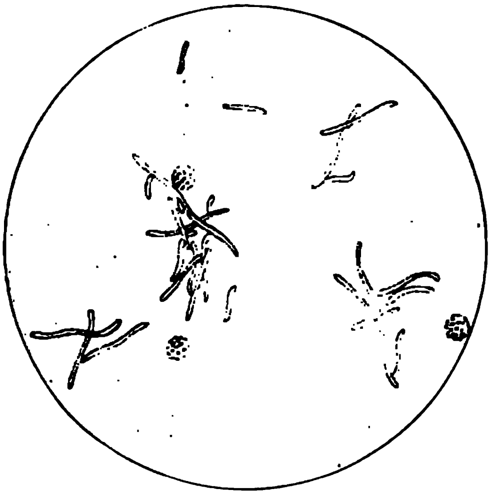

Fig. 46.—Positive Widal reaction; dilution 1-40 (obj. B. and L. one-sixth).

Methods of Making Records.—Number each slide and place under the microscope as soon as they are prepared. Note carefully which, if any, of the above-described conditions are present. Should there be no attempt even at Y formation or clumping, we mark with a wax pencil thus, — (minus), upon the right-hand end of the slide; but in case slight evidence of clumping is present, it is marked — ?; or in case there be present a feeble reaction, agglutination without loss of motion, it is marked + ? (plus). Specimens should be examined every fifteen minutes for a period of one hour, at which time the last marking will be either + or —.

It has been my rule to regard all questionable reactions as being negative, and to mark them —, believing it of less serious moment to err upon this side.

Clinical Significance.—The Widal reaction occurs at some time during the course of typhoid fever in from 95 to 98 per cent. of all cases. The reaction cannot be said to be pathognomonic of this disease, since, rarely, it occurs in other febrile conditions and in jaundice. I have observed the reaction once in the blood of scarlet fever, and again in diphtheria. The reaction may occur early during the course of typhoid fever, but as a rule it is found from the fifth to the ninth day. It may, however, not appear until the twentieth day, or even later when convalescence is well established. The blood of persons who have had typhoid fever may give the reaction for months or years after all other appreciable symptoms of the disease have disappeared. I have obtained a decided reaction—dilution 1-40—thirteen years after an attack of typhoid fever; but other observers have found it to persist over a much longer period. The frequency with which patients are told they have typhoid fever where the condition merely simulates this disease leaves ample room for question whether or not such persons have suffered a mild attack of typhoid at a more recent date. In my own experience I have always questioned the statement of the patient that he had typhoid fever before when the blood gave the Widal reaction three years after typhoid fever. The Widal reaction serves not only in distinguishing typhoid fever from other types of infection, but also in detecting the existence of typhoid infection when it develops co-existent with some other disease.

In the study of a series of 456 cases of suspected typhoid fever in the Philadelphia, the Howard, the Pennsylvania Hospitals, and from private practice the blood was collected upon glazed paper and the dilution approximated 1-40. A specimen collected at the same time and in the same manner was forwarded to the Philadelphia Board of Health for examination. These examinations were made by Dr. A. H. Stewart, who had no knowledge of the fact that I, too, was testing the same blood. Of the 456 cases, 348 were regarded by the clinicians as typhoid fever, and in 341 of these both Dr. Stewart, at the Board of Health, and myself obtained positive Widal reactions. In the seven questionable cases the Board of Health had marked three of them as giving a doubtful reaction, and I had regarded five of them as giving feeble reactions. Whenever the reaction was positive, the clinical course was that of typhoid fever. Such clinical results show the Widal reaction, as practised by the Philadelphia

Board of Health, to be of great importance in the diagnosis of typhoid fever, since of the 456 cases examined but a single specimen was received from any given case. I am not cognizant of such another test of the efficiency of the Widal reaction.

Absence of the Widal reaction in typhoid fever tends toward an unfavorable prognosis, since in non-fatal infection an increase in quantity of agglutinin is to be observed early; therefore agglutination should probably be regarded as significant of a successful step toward protection against the disease. The reaction may vary within wide limitations from day to day. The strength of the dilution, the source of the culture employed in the test, and the temperature at which the bacteria have been grown contribute liberally toward variations in the reaction.

Peculiarities.—Cultures from bile-stained exudates or secretions are liable to display a slight tendency toward agglutination, and the typhoid bacilli are rendered non-agglutinable after exposure for two hours in immune serum that has been deprived of its agglutinative faculty by heating at 75° C. A series of experimental inoculations upon animals is capable of lessening the agglutinative properties of their blood. Agglutination may occur with the blood of infants born of mothers infected with typhoid fever; and rarely, in high dilution, with the blood of healthy adults.

Body Fluids.—Agglutination may take place in connection with such other body fluids as the serum from blisters, pus, exudate from the serous membranes (pleura, pericardium, and synovial sacs), but in these it is not constantly present in a high degree. With the urine of typhoid patients the reaction is usually feeble, may be present from day to day, or may disappear suddenly to again reappear. Clumping is most likely to occur when the urine is employed in high dilution, and the urine of non-infected persons has been known to agglutinate typhoid bacilli. Therefore the reaction with the urine is valueless. The stools of typhoid patients and the milk of both typhoid patients and of animals inoculated experimentally with typhoid bacilli possess the power of agglutination. Passing milk through a porcelain filter deprives it of its clumping powers, as does also a temperature of 120° C. The cerebrospinal fluid (in low dilution, 1 or 3–10), tears, seminal fluid, bile, saliva, and the sweat all possess the agglutinating faculty. Weak solutions of safranin and of vesuvin also agglutinate typhoid bacilli.

Inhibition.—Removal of the salts from the serum by dialysis prevents agglutination, and partial extraction of salts inhibits it. It is not thoroughly understood in what manner agglutination takes place.

There appears to be some question as to whether or not the colon bacillus is agglutinated. Sufficient tangible evidence exists, however, to show that colon bacilli obtained from a variety of sources are agglutinated when placed in typhoid serums; and that the blood of animals immunized to these bacilli agglutinate typhoid bacilli.[*] For description of Ficker's reaction see p. 525.

Paratyphoid Fever.—Other micro-organisms belonging to the colon group, as the Bacillus mesentericus and Bacillus febris gastrica, have been obtained from persons who appeared to be suffering from atypical typhoid fever. They resembled the so-called paratyphoid bacillus, and were found capable of reacting with the blood of patients suffering from paratyphoid.[†]

The bacilli cultivated from the patient's blood (venous) during the course of paratyphoid fever are agglutinated by the blood of the same individual when in high dilution (1–200, Hume; and 1–1000, Brion and Kayser); and by the blood of other persons who are suffering from a like infection. Such bloods do not agglutinate typhoid bacilli even in low dilution. The reaction induced by paratyphoid blood resembles that described under the Widal reaction for typhoid fever.[‡]

Dysentery.—The Bacillus dysentericus (Shiga) reacts with the blood of persons suffering from epidemic dysentery, but there do not appear sufficient convincing reports to enable one to estimate its clinical value.

The work of certain foreign observers and that of Flexner, of New York, has placed this reaction upon a basis where it bids fair to contribute liberally toward both diagnosis and therapy in the disease.

Plague.—Agglutination of the plague bacillus with the serum of persons suffering from this infection has proved of great value in diagnosis. A difficulty in the reaction is that cultures of the plague bacillus are liable to display a mild degree of spontaneous agglutination. The bacilli should be emulsified with a solution of sodium chlorid, and the dilution from 2–1 or 20–1. Serum from convalescents loses its lysogenic action by age, exposure to light, and when heated at 56° C. for a few minutes.[§]

Cholera.—A rather decided reaction is to be displayed between the vibrio of cholera and the serums of infected persons. This reaction has been observed with the serums of persons suffering from cholitis. A positive reaction may be obtained with the filtrate of a forty-eight-hour culture, and agglutination is said to take place when the organisms agglutinated display a lessened virulence. It is also produced by such chemic substances as formalin, safranin, and mercuric chlorid.

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[*] Lommel, "Münchener med. Wochenschr.," 1902, p. 314.

[†] Brion and Kayser, *ibid.*, 1902, p. 611.

[‡] "Jour. Amer. Med. Ass.," Aug., 1902, pp. 187–217.

[§] Row, "Brit. Med. Jour.," May 9, 1903.

Tuberculosis.—Arloing and Courmont, working with an emulsion of a special culture of tubercle bacilli (four weeks old), introduced the culture into the serum of tuberculous persons and animals in dilutions of 1–5, 1–10, and 1–20.*

Twenty-four hours are required for this reaction, and control tubes are necessary. The reaction is present in about 90 per cent. of all cases of tuberculosis; but since it occurs with the blood of 25 per cent. of all healthy persons as well as in non-tuberculous conditions, its clinical value is slight. Tuberculous serum is also capable of agglutinating other bacteria.

Malta Fever.—The serum of persons suffering from Malta fever gives a rather typical reaction with the Micrococcus melitensis when in high dilution (1–50 or greater).

Glanders.—Agglutination of the bacillus of glanders with the serum of infected animals is a fairly constant finding. Characteristic clumping is seen in dilutions of from 1–800 to 1–400.

Pneumonia.—Serum of persons suffering from pneumonia is capable of causing a rather characteristic reaction with a recent culture of the pneumococcus obtained from one ill of pneumonia or from infected animals.

Place the serum in sterile tubes and introduce an equal quantity of a pure culture of the pneumococcus. Incubate at 37° C. for sixteen hours. The reaction consists in the agglutination of the organisms, when they sink to the bottom of the tube. Agglutination becomes more marked with the approach of the crisis and the previously agglutinated diplococci display a distinct capsule.

Bacillus Pyocyaneus.—The serum of immune animals is capable of agglutinating the Bacillus pyocyaneus, dilution 1–50. The serum of animals infected with the Bacillus proteus vulgaris will be found to agglutinate this organism.

Fungi.—It has been shown that the blastomycetes are agglutinated by human serum when in a very high dilution—1–5000 or more,—but it has not been shown that the individual yeast cells are destroyed by this process.†

The precipitates induced by the addition of immune serums to a filtrate of bouillon culture suggest a possible aid to clinical diagnosis. Typhoid bacilli are rendered inagglutinable by dissolution of their capsular envelops. Agglutinin is the term applied to the substance or substances by which agglutination is effected, but its exact nature is not known.

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* "Compt. rend. de l'Acad. des Sci.," Sept. 19, 1898; also "Gazette des Hôpitaux," 1900, p. 137.

† MacFadyean, "Centralbl. f. Bakt.," 1901, vol. xxx, p. 368.

SPECIAL PATHOLOGY OF THE BLOOD.

PERNICIOUS ANEMIA.

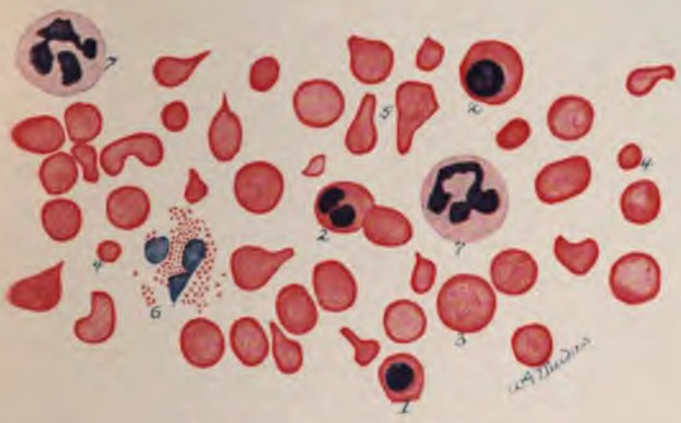
Among various pathologic alterations manifest in the blood of this condition the following occupy prominent positions:

The **oxygen capacity** of the blood is commonly reduced to 50 per cent. of the normal, or less. The hemoglobin is always found to be greatly reduced—40 to 10 per cent.—during the process of blood destruction (active stage), but at the intervals between the attacks the hemoglobin may rise to 50, 65, or 75 per cent., and may remain high for a long period. The specific gravity is low. Each individual erythrocyte is overcharged with hemoglobin (*e. g.*, 1,000,000 red cells and 35 per cent. of hemoglobin—a color-index of 1.75—1,000,000 : 5,000,000 :: 35 : x, and $x = 1.75$; 5,000,000 = the normal number of red cells for 100 per cent. of hemoglobin), although a high color-index has also been observed in leprosy and leukemia. During the stage of remission the color-index is lowered, and with the approaching relapse it increases progressively. Exceptionally, I have seen the color-index remain high throughout the stage of remission, and according to some observers it may exceed that of the active stage.

Significance.—A high color-index should probably be regarded of bad prognostic value, while a low index and an average decrease in the diameter of the erythrocytes to that of the normal are usually observed during the decline of the active stage, and may continue during the greater part of the intermission. The red cells increase rapidly from 1,000,000 to 2,000,000 (active stage) to 3,000,000 to 4,000,000, or more. The hemoglobin may be equally distributed among the cells (see *Degeneration*, page 88); and the microcytes are charged with hemoglobin beyond their normal capacity, while the amount of hemoglobin occupying the larger cells may not be in excess when taken in correlation with their increased diameters.

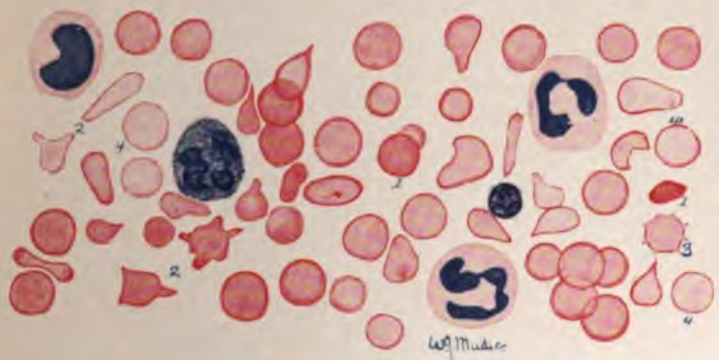
Active Stage.—Between 50 and 75 per cent. of the erythrocytes show diameters decidedly above that of the normal red cell. The presence of microcytes often reduces the average cell-diameter to nearly that of the normal, and there is often a tendency for

PLATE 6.



BLOOD OF PERNICIOUS ANEMIA.

1, Normoblast; 2, megaloblasts, one showing two nuclei; 3, macrocyte; 4, microcyte; 5, poikilocytes; 6, ruptured eosinophiles; 7, polymorphonuclear leukocytes (specimen stained with eosin and hematoxylin Obj. Spencer one-twelfth oil-immersion).



BLOOD OF CHLOROSIS.

1, Normal-sized erythrocyte stained deeply; 2, poikilocyte; 3, crenated erythrocyte; 4, normal cells stained feebly. Note basophilic leukocyte in left half of plate. All red cells stain less deeply than do the corresponding cells in pernicious anemia. (Specimen is stained with eosin-hematoxylin and methylene-blue. Obj. Spencer one-twelfth oil-immersion).

the cells to become oval or elliptic in outline (Plate 6). Poikilocytosis is marked, and many of the necrobiotic changes (polychromatophilia, etc.) described on page 91 are to be found in the stained specimens.

Nucleated Red Cells.—Nucleated red cells are commonly found. Megaloblasts appear to bear more significance in pernicious anemia than when found in the anemia of intestinal parasites or elsewhere. Normoblasts and microblasts are common findings, and I have found the number of nucleated red cells per cubic millimeter to vary from 2 to 950. Cabot, however, has found 7000 nucleated erythrocytes per cubic millimeter.

During the active stage leukopenia is a common occurrence. The decrease in the number of leukocytes concerns mostly the polymorphonuclear cells, and to this reduction there is a corresponding increase in the percentage of lymphocytes (30 to 70 per cent.), yet an absolute lymphocytosis does not exist. A mild grade of eosinophilia and from 3 to 9 per cent. of myelocytes may be seen.

Remission.—During the stage of remission a moderate leukocytosis may exist, such increase concerning the polymorphonuclear neutrophils, and coincident with it the percentage of lymphocytes decreases and the myelocytes disappear. Rare, indeed, it is to see cases of this disease wherein leukocytosis and splenic enlargement with a rather high percentage of myelocytes are displayed, but their percentage does not equal that of leukemia.

Significance of the Blood Findings.—

FATAL ISSUE EARLY.	PROTRACTED COURSE.
1. Progressive anemia; interval of remission short or absent.	1. Remissions frequent and of long duration.
2. Color-index high.	2. Color-index slightly increased.
3. Increase in size of red cells, tendency toward oval and elliptic forms.	3. Red cells of normal size, or many cells under size.
4. Marked degenerative changes in the erythrocytes.	4. Little degeneration present.
5. Megaloblasts numerous — exceed normoblasts.	5. Megaloblasts few. Normoblasts plentiful.
6. Decrease in polymorphonuclear leukocytes.	6. Percentage of polymorphonuclear cells normal.
7. Lymphocytosis present.	7. Absent.

CHLOROSIS.

The chlorotic type of anemia is characterized by an increase in the volume of blood, decided fluidity, pallor of the drop, a tendency toward rapid coagulation, reduction in the percentage of hemoglobin, and a low color-index; *e. g.*, 3,500,000 red cells, 40 per cent. of hemoglobin, and a color-index of 0.57. The red cells are slightly undersized, pale, and vary from 3,500,000 to 4,500,000 per cubic millimeter and rarely they fall to 2,000,000. The stained blood is less apt to show disseminated areas of pallor, polychromatophilia, and punctate basophilia than is the blood of either pernicious anemia or of grave secondary anemia (Plate 6), but poikilocytosis is well marked. Normoblasts are encountered, and the blood-plates usually increased. Leukocytosis is absent in uncomplicated cases, while there is apt to be an absolute reduction in the polymorphonuclear cells. Moderate lymphocytosis (30 to 50 per cent.) occurs, wherein either the large or small lymphocytes may predominate. Eosinophilia is a rare symptom, and myelocytes have been observed.

Hydremia.—According to the view of Smith, the volume of blood may be one-half or more greater than that of normal, and there is an absolute increase in the number of both red and white cells, but such increases are masked by the excess of plasma. Smith further believes that treatment decreases the plasma, and thus restores the normal condition. These views, however, are not universally accepted.

Specific Gravity.—The specific gravity is in direct correlation to the hemoglobin, and its estimation is often of the utmost clinical value, since it may fall to 1.025 to 1.035 (1.059 to 1.060 normal).

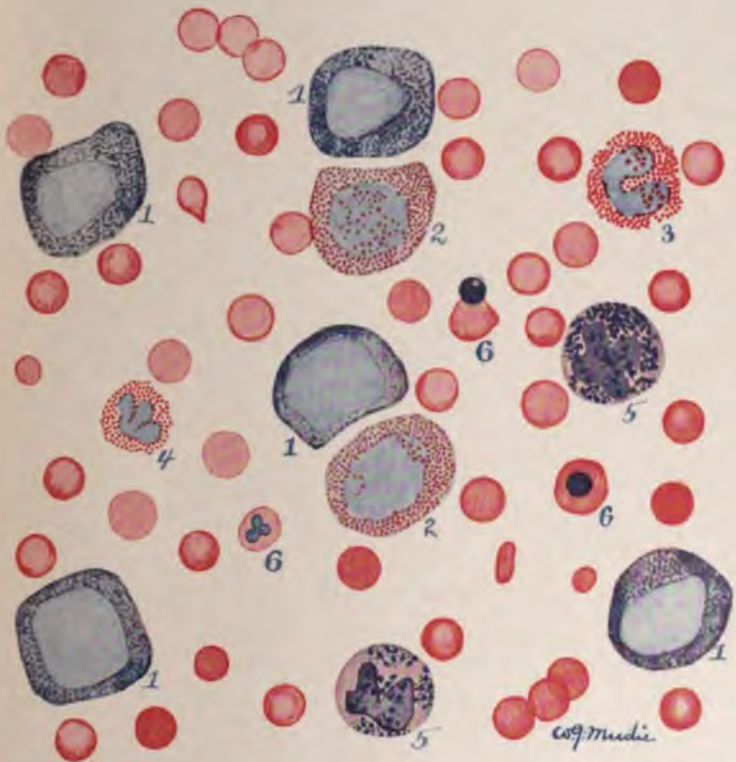
Nucleated Erythrocytes.—Normoblasts are occasionally detected in chlorotic blood, and their number increases with the severity of the condition.

During convalescence the red cells are increased, and later the coloring-matter and specific gravity return to normal. Secondary anemia without leukocytosis may be indistinguishable from chlorosis.

TABLE DISTINGUISHING HEMATOLOGIC FEATURES BETWEEN PERNICIOUS ANEMIA, CHLOROSIS, AND SECONDARY ANEMIA.

Pernicious Anemia.	Chlorosis.	Secondary Anemia.
1. Disproportionate reduction in the hemoglobin and red cells; high color-index.	1. Decided reduction of hemoglobin ; color-index low	1. Hemoglobin and red cells reduced proportionately; color-index about normal.
2. Red cells may fall to 1,000,000 per cubic millimeter or lower during the active stage.	2. Usually 3,500,000 to 4 500,000.	2. Rarely below 2,000,000.
3. Leukocytosis absent; leukopenia the rule.	3. Absent.	3. Presence or absence not diagnostic.

PLATE 7.



BLOOD OF MYELOGENIC LEUKEMIA.

1, Myelocytes; 2, eosinophilic myelocytes; 3, eosinophile; 5, mast-cell; 6. nucleated erythrocytes, one with clover-leaf nucleus and one with nucleus on cell's margin (stained with Jenner's stain. Obj. B. and L. one-twelfth oil-immersion).

PERNICIOUS ANEMIA.	CHLOROSIS.	SECONDARY ANEMIA.
4. Lymphocytosis; polynuclear cells reduced.	4. Lymphocytosis; polynuclear elements decreased.	4. Increase affects mostly the polynuclear cells.
5. Eosinophilia absent.	5. Absent.	5. Present under certain conditions.
6. Myelocytes few.	6. Extremely rare.	6. Occasionally seen in varying numbers.
7. Diameter of red cells increased; oval and elliptic forms common.	7. Diameter decreased or normal.	7. Most cells of normal size.
8. Erythrocytes stain deeply.	8. Stain feebly—many as mere shadows.	8. Appear normal or moderately pale.
9. Megaloblasts exceed in number the normoblasts.	9. Normoblasts few.	9. Normoblasts present; megaloblasts rare.
10. Specific gravity low.	10. Reduction in proportion to loss of hemoglobin.	10. Reduced — follows course of hemoglobin.
11. Not materially altered.	11. Blood-plates increased.	11. Fluctuate greatly.

Difficulties.—It is not uncommon to meet cases wherein one is unable to decide hematologically to which of these three types of anemia the case belongs; and here the diagnosis is to be achieved through a comparative study of both the general clinical features and the blood findings. Obscure syphilis, gastric carcinoma, intestinal parasites, purpura, malaria, typhoid fever, and less often mineral poisons may be found to produce changes in the blood which closely resemble those found in pernicious anemia. Except in anemia of parasitic origin megaloblasts are seldom found.

LEUKEMIA.

Leukemia is a disease characterized by hyperleukocytosis, a proportionate reduction in both the hemoglobin and the red cells, the occurrence of nucleated erythrocytes, and the presence of myelocytes. Generally speaking, leukemia may be divided into two classes, myeloid and lymphoid. Myeloid blood is found when there exist hypertrophy of the spleen and marrow changes with little or no involvement of the other lymphatic tissues. Lymphoid blood may be associated with acute or chronic forms of this disease, and occurs when some of the lymphatic glands are enlarged, though visible glandular enlargements may be absent, as was noted in the case from whom the blood was obtained for Plate 9. There may be enlargement of the spleen. Both types of the disease (mixed leukemia) may exist in the same individual. Leukemia is one of the few conditions wherein we are able to make a diagnosis from the hematologic findings alone.

MYELOID LEUKEMIA.

The blood exudes slowly from the puncture, is opaque, may resemble thin pus, and spreads upon slides with difficulty. The fibrin and the time for coagulation are about normal.

Red Cells.—During the early stage the red cells are nearly normal in number, but with the advance of the disease they are reduced to from 3,500,000 to 2,000,000 or less. I have seen the red cells remain near the normal point until late in the disease. The hemoglobin is also reduced, giving a color-index of from 0.5 to 0.6. Nucleated red cells are to be found in great numbers independent of the degree of anemia, varying as they do from 5000 to 50,000 or more per cubic millimeter. The majority of these cells are normoblasts, and many show fragmentation and mitosis of their nuclei (Fig. 47). Other changes in the red cells common to grave anemia are also prominent in myeloid blood. Polychromatophilic erythroblasts are commonly seen (Plate 8) and were present in all of 16 cases that have come under my observation.

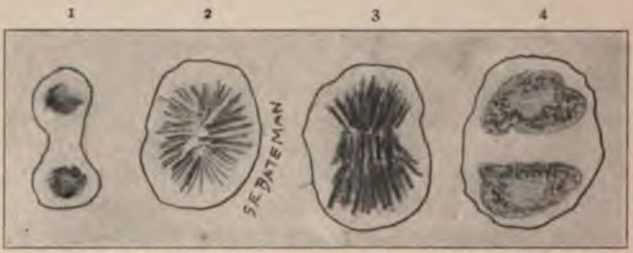
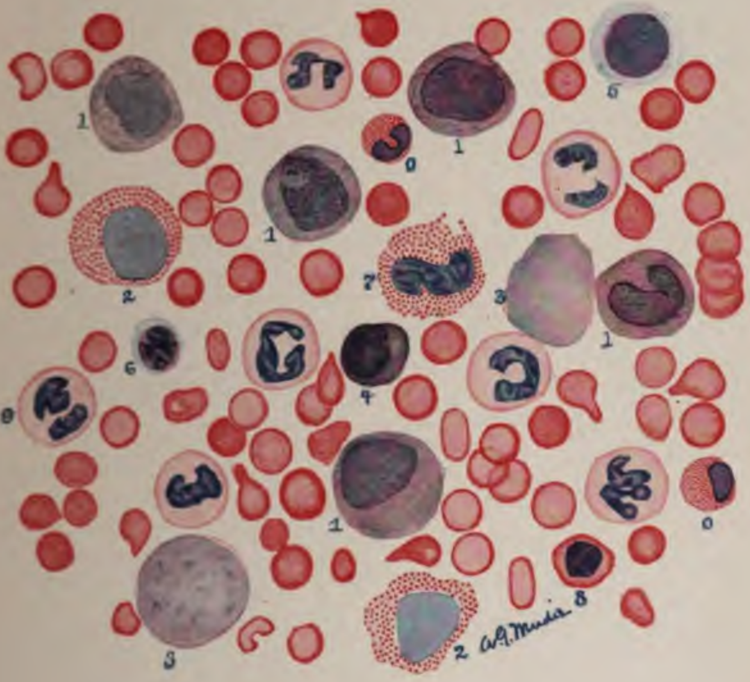


Fig. 47.—1, Mitosis of red corpuscle from leukemic blood; 2, myelocyte from same blood; 3, megaloblast from case of fatal anemia; 4, myelocyte from leukemic blood (after George Dock).

White Cells.—At first the white cells may vary from 60,000 to 100,000 per cubic millimeter, but later they are found to number about 400,000 to 500,000, and may reach 1,000,000 per cubic millimeter. Correlatively speaking, the leukocytes and red cells may be found 1:100, 1:50, or even 1:10, fluctuating during different hours of the day. The fresh blood is found to contain many leukocytes, some of which show ameboid movement (myelocyte). In one instance (personal observation) with a leukocyte count of 550,000 per cubic millimeter and 34 per cent. of myelocytes all symptoms were modified and the leukocyte count fell within a period of six weeks to 25,000, myelocytes disappeared, and the patient was reported by another observer as cured. Cabot cites a similar instance. As a rule, however, during the stage of remission the percentage of myelocytes remains high.

PLATE 8.



Blood of Splenomedullary Leukemia.

1, Myelocytes; 2, eosinophilic myelocyte; 3, leukocytic shadows; 4, polychromatophilic megaloblast; 5, large mononuclear leukocyte; 6, small lymphocyte; 7, eosinophile; 8, megaloblast; 9, polymorphonuclear leukocyte; 10, small eosinophiles (stained with eosin and hematoxylin. Obj. B. and L. one-twelfth oil-immersion).

Myelocytes.—The distinguishing feature of myeloid blood is the high percentage of myelocytes present—20 to 60 per cent. (Plate 8) (see *Myelocytes*, page 97). There is an absolute increase in the number of polynuclear cells, though their percentage upon differential count is somewhat reduced. They are seen to vary within wide limits as to size, staining properties, and peculiarities of their nuclei, which constitute an important feature in leukemic blood (dwarfed cells, giant cells, cells containing coarse granules). Border-line cells between the myelocyte and the polynuclear leukocyte are frequently encountered; Cabot prefers to classify such cells as transitional neutrophils. The lymphocytes usually occupy from 20 to 30 per cent. of the total number of leukocytes, but they, too, show an absolute increase, and the percentage of large and small forms varies greatly in different cases. Large lymphocytes which stain not unlike myelocytes are often seen; and cells, apparently small lymphocytes, display granules.

The eosinophils show an absolute increase and their percentage may vary from 1 to 15 or more. In leukemia there are to be distinguished polynuclear eosinophils of normal size, eosinophilic giant cells often containing coarse granules, dwarfed eosinophils, and the eosinophilic myelocyte (see Plate 8). Eosinophils at times contain basophilic granules.

Basophils.—Blood stained with thionin shows neutrophils with coarse granules (Mastzellen). Such cells may comprise 10 per cent. of the leukocytes (Plate 7). Charcot-Leyden crystals are to be found in myeloid blood and in blood containing many eosinophilic cells.

LYMPHATIC LEUKEMIA.

Lymphoid blood is characterized by a large percentage of lymphocytes (70 to 93). This form of leukemia is commonly acute, yet it may run a protracted course. In three cases that have come under my observation the leukocyte counts have varied from 30,000 to 450,000 per cubic millimeter, yet a higher degree of leukocytosis may be observed, and the red cells vary from 3,500,000 to 1,200,000 per cubic millimeter. Nucleated red cells are less commonly met with in this condition than in myelogenous leukemia. McCrea, in the study of 11 cases occurring in infants, did not find nucleated red cells in the blood of 7 of them; but in acute cases nucleated red cells are occasionally numerous, and were present in all of my cases. Degenerative changes in the red cells are fairly pronounced. The ratio of white to red cells has been seen to equal 1 : 50 or less. The large percentage of lymphocytes may be composed either of the small forms, the large forms, or of both. In one of my cases there was such a gradation in the cells as to make it impossible to distinguish between the large and small lymphocytes (Plate 9). In acute cases the large cells often predominate; such cells stain faintly and show evidence of degeneration, and occasionally stain a pinkish color. In specimens displaying mostly small lymphocytes these cells stain deeply (Plate 9).

CHARACTERISTIC DIFFERENCES IN LEUKEMIC BLOOD.

Myelogenic Leukemia.	Chronic Lymphatic Leukemia.	Acute Lymphatic Leukemia.
1. Red cells moderately reduced—3,000,000.	1. Moderate reduction—2,000,000 to 3,000,000.	1. More marked reduction early during course of malady.
2. Nucleated red cells, including megaloblasts, numerous.	2. Rare.	2. Normoblasts rather common.
3. Leukocytes 150,000 to 500,000 or more per cubic millimeter.	3. 100,000 to 300,000.	3. 30,000 to 200,000.
4. Myelocytes 30 per cent. or more.	4. Myelocytes few.	4. Myelocytes few.
5. Eosinophils common, also eosinophilic myelocytes.	5. Eosinophils few	5. Eosinophils rarely found.
6. Neutrophils commonly seen.	6. Less common.	6. Scanty.
7. Lymphocytes — large and small—20 to 30 per cent.	7. Small lymphocytes 70 to 90 per cent.	7. Lymphocytosis—large forms predominant.

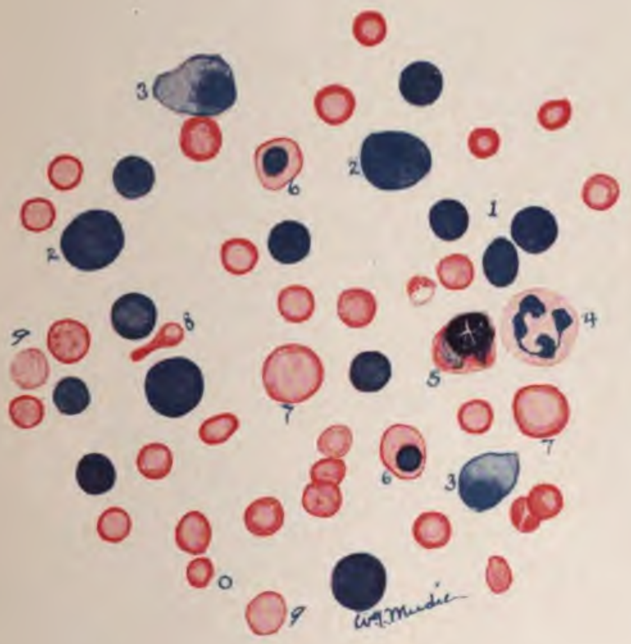
PSEUDOLEUKEMIA (Hodgkin's Disease).

Pseudoleukemia is distinguished from true leukemia through hematologic research only. Early during the course of this disease the red cells are practically normal, and with its progress the hemoglobin falls gradually (75 to 30 per cent.), and is soon followed by a reduction of the red cells. Should these changes develop rapidly they are accompanied by the general features of grave secondary anemia. The leukocytes may vary from 5000 to 60,000 per cubic millimeter, and may increase to a ratio of 1 : 50 or even 1 : 80 of the red cells, the polynuclear cells alone being increased. In cases where the leukocyte count is low lymphocytosis is often found. Eosinophilia is seldom seen and these cells are usually decreased when leukocytosis exists. Similar blood changes are to be found in glandular tuberculosis, malignancy, and syphilis.

SPLENIC ANEMIA.

A condition characterized by splenic enlargement and leukopenia. The red cells are usually between 2,000,000 and 4,000,-

PLATE 9.



BLOOD OF LYMPHATIC LEUKEMIA.

1, Small lymphocytes; 2, large lymphocytes; 3, degenerated lymphocytes; 4, polymorphonuclear leukocytes; 5, megaloblast showing fragmentation of nucleus and polychromatophilia of protoplasm; 6, nucleated red cells (megaloblasts); 7, macrocytes; 8, poikilocyte; 9, erythrocytes of normal size; 0, microcyte (stained with eosin and hematoxylin. Obj. Spencer one-twelfth oil-immersion).

000 per cubic millimeter. White cells may fall to 700; while the hemoglobin fluctuates between 20 and 50 per cent. Myelocytes and normoblasts are scanty, and moderate lymphocytosis the rule.

ACUTE INFECTIOUS DISEASES.

The blood changes common to infectious fevers, as the result of temperature alone, have been alluded to (p. 108).

Pneumonia.—Coagulation is rapid, except in cases where leukopenia exists. Variations in the specific gravity and polycythemia result from cyanosis. In children the specific gravity may be high.* The toxicity of the blood is increased, and after the crisis both hemoglobin and red cells are reduced. Normoblasts and megaloblasts may be found.

Leukocytosis develops with the onset and persists, declining with the approach of the true crisis. The polymorphonuclear leukocytes are both absolutely and relatively increased (90 per cent.), and the lymphocytes are reduced, as are also the eosinophils, before the crisis; but later they are increased. Myelocytes and transitional neutrophils are common. The blood-plates are increased following the crisis. When leukocytosis continues after the crisis, resolution is retarded, and such complications as empyema, abscess, and gangrene cause a further increase. Children show high leukocyte counts.

Significance.—Slight leukocytosis—mild infection. High leukocytosis—moderate or severe infection. Absence of leukocytosis—severe infection. In 16 fatal cases Rosenow† found the leukocytes to average 12,000; while 40 cases of recovery gave an average of 20,000.

Typhoid Fever.—During the course of this disease the blood findings may vary greatly, dependent upon the existence of profuse diarrhea, constipation, pyrexia, hemorrhage, peritonitis, pneumonia, etc. (see *Bacteriology of the Blood* and *Widal Reaction*). Blood-plates and fibrin are commonly reduced—a great reduction being of rather bad prognosis. Leukocytosis is not a feature of typhoid fever, the average being about 5000 per cubic millimeter, and leukopenia at times progresses with the advancing stages of the disease. Leukocytosis may or may not follow hemorrhage from the bowels, and is liable to develop with complications, as well as when the ulceration of the bowel is sufficiently deep to produce local peritonitis. Thayer gives the following table showing the percentage of leukocytes found by differential counts:

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* Monti and Berggrund, "Arch. f. Kinderheilk.," vol. xvii.

† "Jour. Amer. Med. Assoc.," March 18, 1905, p. 872.

	Counts.	Polymorphonuclear Neutrophils. Per Cent.	Small Mononuclear. Per Cent.	Large Mononuclear. Per Cent.	Eosinophils. Per Cent.
First week of fever	12	74	13	12	0.5
Second " " "	39	71	14	13	0.8
Third " " "	34	66	21	11	0.3
Fourth " " "	19	65	20	14	0.4
Fifth " " "	8	62	18	19	0.3
Sixth " " "	4	58	22	13	6.0
First week apyrexia	12	61	21	15	3.0
Second " "	7	49	31	17	2.3
Third " "	6	57	15	23	3.5

Osler* cites a case wherein the leukocytes were 5500 before intestinal perforation, and rose to 11,500 within fifteen minutes following this accident; but about three and one-half hours later they were found to be 7500, and six hours after perforation they numbered 7700. I have been unable to find definite records wherein the leukocytes were counted just preceding intestinal perforation and at regular intervals after its occurrence. In the cases I have studied and in a number reviewed in the literature a gradual progressive increase in the number of leukocytes often followed perforation; but a fact to be borne in mind is that perforation may have taken place some hours before the initial counts were made, and for this reason particularly I hesitate to accept without question the view that leukocytosis is found during the first six hours following intestinal perforation.

The leukocytes are influenced by treatment, as hypodermoclysis, cold baths, drugs, etc. (page 101). The eosinophils are decreased or absent during the febrile period, and degeneration of the leukocytes, leukocytic shadows, and leukocytes showing granules of glycogen are to be seen.

During the febrile period a progressive diminution in both the number and percentage of the polynuclear cells occurs, with a corresponding increase in the lymphocytes and large mononuclear elements. Eosinophils are diminished or absent, but increase as convalescence is established. The percentage of polymorphonuclear cells may remain stationary where complications and leukocytosis are present. Generally speaking, the specific gravity, hemoglobin, and red cells show the changes common to secondary anemia. Among the favorable signs Nagaeli places a decided decrease in all forms of leukocytes and the absence of leukocytosis during the existence of complications. Bacteremia may develop. No clinical importance is attached to the recovery of typhoid bacilli from the rose spots, since they may be cultivated from the skin and perspiration.

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* "Trans. Phila. Co. Med. Soc.," Feb., 1901.

Diphtheria.—The red cells remain normal or above normal, except during convalescence, when they show moderate reduction. A slight reduction follows the administration of antitoxin. The hemoglobin is but slightly reduced—5 to 10 per cent. Billings* found the diminution less marked in cases treated with antitoxin. Anemia and bacteremia may, however, develop (see *Bacteriology of the Blood*).

Leukocytosis.—Leukocytosis is an early feature in diphtheria and is, as a rule, in proportion to the severity of the disease, 15,000 to 30,000 per cubic millimeter. It may reach its height the second day (severe type) or not until the sixth day, while malignant cases often show progressive leukocytosis or leukocytosis may be wanting. Other throat disorders with equally marked symptoms show, as a rule, a higher leukocyte count than does diphtheria. The polynuclear neutrophil cells are increased and a relative or, at times, actual lymphocytosis may be found. Eosinophils are decreased or normal. Myelocytes are present, and should they exceed 3 per cent., are of grave prognostic moment. "Leukocytic shadows" are common findings, and Ewing demonstrated an increased acidophile tendency of the neutrophil granules.

Scarlet Fever.—The red cells are moderately reduced (3,000,000 to 4,000,000 per cubic millimeter) during convalescence, and hemoglobinemia has been observed. There is some distortion of the red cells, and normoblasts are occasionally seen. Bacteremia may ensue (see *Bacteriology of the Blood*).

White Cells.—Leukocytosis is to be observed early (15,000 to 30,000 per cubic millimeter), and even before the appearance of the eruption, falling with the decline of temperature; but rarely it persists for days or weeks after the temperature is normal. Over 40,000 leukocytes is of bad prognostic omen. The polymorphonuclear cells are increased (80 to 90 per cent.), early returning to the normal in favorable cases.

Characteristic Feature.—Eosinophilia is present (5 per cent.) in all except malignant cases, and persists during convalescence until the sixth week, when it may reach from 10 to 20 per cent. Eosinophilia is of serious prognostic value in scarlatinal nephritis (Neusser), and when absent in scarlet fever myelocytes are to be found.

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* "Johns Hopkins Hosp. Bul.," 1894, p. 105.

Measles.—The fibrin is increased when decided inflammation of the mucous surfaces is present. The red cells are practically normal; and leukopenia may be encountered during the eruptive stage. Both the lymphocytes and large mononuclear cells are increased during convalescence. The eosinophils are normal or diminished during the febrile stage. Cabot found 6000 and 8000 leukocytes per cubic millimeter in two cases of German measles. Weber* recovered a protozoon from the blood of measles.

DISTINGUISHING HEMATOLOGIC FEATURES OF DIPHTHERIA, SCARLET FEVER, AND MEASLES

Diphtheria.	Scarlet Fever.	Measles.
1. Leukocytes, 25,000 to 30,000 and in severe and fatal cases these cells may number 50,000 per cubic millimeter.	1. Leukocytes, 15,000 to 30,000 or 40,000 at the onset and before the appearance of the eruption.	1. Leukocytes absent; leukopenia the rule.
2. A relative lymphocytosis common; the polynuclear cells are moderately increased.	2. Polynuclear cells 85 to 95 per cent.; lymphocytes normal or subnormal.	2. Lymphocytes and large mononuclears are increased during convalescence.
3. Eosinophils of normal ratio, decreased, or absent.	3. Eosinophils increased after second or third day; 10 to 15 per cent. by the second week; return to normal the sixth week.	3. Eosinophils diminished the rule.
4. Myelocytes present, 1 to 4 per cent.	4. Myelocytes unusual findings.	4. Absent.
5. Leukocytic shadows common.	5. Rare.	5. Rare or absent.

Small-pox.—During the febrile stage of small-pox polycythemia may prevail, and later, during convalescence, a mild grade of chlorotic anemia develop. According to Pick's painstaking research cases of small-pox with moderate eruption showed the leukocytes to be normal or subnormal in number. Leukocytosis develops as a result of suppuration, and may oscillate between 12,000 and 20,000 or more per cubic millimeter, increasing relatively with the advancing suppuration. In one instance I found 30,000 leukocytes per cubic millimeter six weeks after convalescence had been established. Pneumonia as a complication is coupled with a reduction in the number of leukocytes. Ameboid bodies have been detected in the blood-cells of small-pox, measles, and scarlet fever, and have been regarded by Weber and Doehle and by a number of other observers as developmental stages of a protozoon. See Exudates, pp. 492 and 499.

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* Centralbl. f. Bakt.," vol. xxi, No. 6.

Vaccinia.—Sobotka (quoted by Ewing) found the red cells and hemoglobin to be normal after vaccination. Leukocytosis develops on the third or fourth day following inoculation; after which a daily fall to the seventh or eighth day ensues, resulting in leukopenia (height of fever). From the tenth to the twelfth day secondary leukocytosis develops. In Sobotka's observations the primary leukocytosis ranged between 12,000 and 23,000 cells per cubic millimeter, and the secondary leukocytosis from 10,000 to 17,500.

Suppuration (*Septicemia, Pyemia, Purulent Cystitis, Osteomyelitis, Abscesses, etc.*).—In conjunction with suppurative processes wherein the products of suppuration are not firmly encapsulated within the human organism leukocytosis develops in direct correlation with the degree of infection; such increase affecting, for the most part, the polymorphonuclear cells. Leukocytosis, therefore, together with other clinical findings may be invaluable; but its absence does not exclude the existence of suppuration. Acute inflammatory processes also induce leukocytosis; while suppuration of mucous surfaces may excite but slight leukocytosis.

Both the hemoglobin and the red cells are reduced in keeping with the degree of suppuration; but the hemoglobin is more sensitive than the red cells and its loss greater (color-index low). The erythrocytes present the changes common to secondary anemia.

Absence of Leukocytosis.—Asthenic cases of septicemia may not show leukocytosis and, in fact, may display leukopenia.

Chemistry.—The hemoglobin at times appears to enter into solution with the plasma (hemoglobinemia) when there has been great blood destruction. There may also be a tendency toward crystallization of the hemoglobin, which is detected in the fresh blood by crystals forming about the margin of the cover-glass. The specific gravity is commonly reduced, and the albumins are lowered in proportion to the severity of the infection. Glycogen is a rare finding. (For bacteriologic findings see page 113.)

Appendicitis.—In this disease there appear to be no hematologic findings that are of great clinical value. A purely catarrhal appendicitis is probably seldom placarded by leukocytosis; while the majority of severe cases show leukocytosis ranging from 12,000 to 25,000 cells per cubic millimeter between the second and fourth days. Leukocytosis may be wanting in instances where suppuration exists, or vice versâ. Judging from the literature (including researches of Cabot and of Deaver and Da Costa) and from my own observations upon a rather large series of cases, the hematologic findings furnish but little aid in the diagnosis of appendicitis.

Erysipelas.—During the course of erysipelas leukocytosis is an almost constant symptom, and in many instances is directly proportionate with the type and extension of the infection. In fifteen cases examined at the Philadelphia Hospital the leukocytes numbered from 10,000 to 23,000 per cubic millimeter upon the patient's admission. The polynuclear cells are apt to be influenced mostly, and the eosinophils are likely to be diminished or absent. Bacteriologic study of the blood has proved of no clinical value.

Acute Rheumatism.—In this disease the red cells are liable to moderate reduction while the hemoglobin suffers a greater loss, falling to 55 to 75 per cent., and during convalescence the return of the red cells to the normal precedes that of the hemoglobin.

Leukocytosis.—A leukocyte count of from 10,000 to 15,000 cells per cubic millimeter represents an average case, but there are recorded instances wherein high-grade leukocytosis existed in connection with such complications as pericarditis, endocarditis, and pneumonia. The proportional relation of the various leukocytes is rather well maintained in uncomplicated cases; the eosinophils, however, are absent at the onset, present during the disease, and increased in convalescence.

Whooping-cough.—Whooping-cough serves as the only example of an afebrile disease affecting the respiratory tract wherein decided leukocytosis is a constant symptom. The degree of leukocytosis is influenced by the age of the child, and is found to range from 20,000 to 40,000 cells per cubic millimeter in uncomplicated cases during the active stage; the lymphocytes may range between 35 and 55 per cent.; the polynuclear cells are relatively decreased; and eosinophils normal or diminished. The hemoglobin and red cells bear no direct relation to the degree of leukocytosis.

Tonsillitis and Serous Effusions.—Tonsillitis shows a leukocytosis of from 10,000 to 20,000 per cubic millimeter, as do also effusions into the serous membranes during the febrile stage; and such effusions when they have become purulent show a comparative increase in the number of leukocytes. Purulent peritonitis may exist without leukocytosis, and when the exudate ceases to be poured out, leukocytosis is liable to disappear. Effusions excited by the tubercle bacillus are infrequently accompanied by leukocytosis.

Influenza.—The most peculiar feature of this disease is that it is of bacterial origin, ushered in with a chill, and does ***not***

show leukocytosis. On the contrary, uncomplicated cases are liable to display leukopenia; but when complications arise, leukocytosis may be encountered. There are no constant changes to be observed in either the red cells or the hemoglobin. I was unable to recover Pfeiffer's bacillus from the venous blood examined at various stages of the disease (see *Bacteriology of the Blood* and *Serum-diagnosis*, pages 113, 116).

Bubonic Plague.—It is conceded by all observers that leukocytosis is present both during the active stage of this disease and throughout convalescence, varying from 20,000 to 50,000 or more. Of these, the polynuclear cells are mostly concerned, and next in order are the lymphocytes. The eosinophils are normal or decreased (see *Serum-diagnosis* and *Bacteriology of the Blood*, pages 114, 124).

Malta Fever.—In addition to the agglutinative properties of the serum in this disease (see *Serum-diagnosis*, page 125) Musser and Sailer* have found the red cells to be normal in number, the leukocytes slightly increased, and the hemoglobin reduced to 60 per cent.

Yellow Fever.—In this condition the globulicidal properties of the serum are increased, the red cells approximately normal, while the hemoglobin suffers severe destruction, falling to from 75 to 50 per cent. Leukocytosis may be present; eosinophilic cells are normal or reduced; and myelocytes rarely found. Ewing refers to two cases showing hemoglobinemia.

Gonorrhea.—Uncomplicated cases of gonorrheal urethritis exert slight influence, if any, upon the red cells or the hemoglobin, but in the presence of complications these may be affected. Leukocytosis is likewise produced by complications and may obtain in uncomplicated cases. Extension of the inflammatory process to the deep urethra, epididymis, etc., may induce a high percentage of eosinophils. Occasionally the gonococcus invades the circulation (see *Bacteriology of the Blood*, page 114).

Actinomycosis.—The pulmonary form of this disease may show 20,000 or more leukocytes per cubic millimeter, as exemplified by Ewing's case.† A chlorotic form of anemia has been observed by Bierfreund.

Anthrax and Glanders.—Bacteremia rarely develops during the course of anthrax (see *Bacteriology of the Blood*, page 111), and I have been unable to discover from the literature any data as to the condition of the red cells, hemoglobin, and leukocytes in this disease. Cabot has found leukocytosis—11,600 to 13,600—in a case of glanders.

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* "Phila. Med. Jour.," 1898 p. 1408.

† "Clin. Path. of the Blood," first ed., p. 291.

Leprosy.—During the various stages of leprosy polycythemia may be found, and is usually ascribable to local stasis (cyanosis). Winiarski, in the study of 17 cases, found the red cells above 6,000,000 per cubic millimeter. He also found 2,300,000 and 1,900,000 cells in two well-advanced cases. The hemoglobin ranged between 80 and 118 per cent. in all but the two cases showing a low number of red cells. The leukocytes were normal or subnormal in number, and the lymphocytes increased, maximum of 47 per cent.; but lymphocytosis did not exist in connection with suppuration. Brown obtained similar results in 16 cases.

In a case studied at the Medico-Chirurgical Hospital, through the courtesy of Dr. J. V. Shoemaker, I found the hemoglobin 65 per cent., red cells 3,460,000, white cells 6800, per cubic millimeter. The erythrocytes were deficient in color, staining feebly as a rule, though many of the smaller cells stained deeply. Decided variability in size and form of the red cells existed, among which macrocytes were predominant.

Nucleated Red Cells.—While counting 200 leukocytes, 40 nucleated red cells were found, and of these 30 were normoblasts and 10 megaloblasts; many of the latter variety showed well-marked polychromatophilic properties. A differential leukocyte count gave polymorphonuclears 60.2, transitionals 13.9, eosinophils 8.7, small lymphocytes 4.3, large lymphocytes 9.5, large mononuclears 3.4 per cent. Leukocytosis was not present in the cases examined by Brown or those examined by Winiarski.

Bacilli.—Lepra bacilli were detected in the circulating blood in my case* (see *Bacteriology of the Blood*, page 112).

Takosis.—A contagious disease of goats which is excited by a diplococcus (Micrococcus caprinus) which invades the tissues and is found in the blood during the various stages of the disease. J. R. Mohler and H. J. Washburn† describe the blood findings in this condition. Smears made from the peripheral blood may show the diplococcus, which is always detected in the plasma and never situated within the blood-cells. Cultures obtained from the peripheral and venous circulations antemortem and from the heart's blood and the viscera at postmortem will be found to develop the Micrococcus caprinus. Persons who skin animals dead of takosis become infected and develop initial blebs and abscesses. The Micrococcus caprinus is found in the exudates from these lesions.

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*"Proc. Phila. Co. Med. Soc.," Jan., 1903.

†"Bureau of Animal Industry Bul.," 1903, No. 45.

The number of erythrocytes are increased from one to two millions per cubic millimeter (9,000,000 to 10,000,000 normal for the sheep); and the leukocytes are increased from 9000 to 10,000 (normal) to 18,000 to 20,000, which increase affects the polymorphonuclear and eosinophilic cells.

Hemocytolysis.—In this condition the isotonic tension of the plasma may be reduced below that of the red cells, when the hemoglobin is liable to be extracted from the erythrocytes and dissolved in the plasma, leaving a mere shadow of a once deeply colored red cell. Again, without appreciable diminution in the isotonic tension of the plasma, the erythrocytes undergo disintegration and are broken into fragments. The injection of a large quantity of normal salt solution into the circulation may excite the former condition, while virulent blood poisons produce the latter. The two conditions, however, often go hand-in-hand, and are to be seen in diverse degrees in various types of anemia. Among the special conditions wherein the blood-cells suffer such rapid and extensive destruction should be mentioned high temperature, poisoning by guaiacol, nitroglycerin, amyl nitrite, nitrobenzol, fumes of arseniureted hydrogen, snake venom, and the ingestion of toad-stools. All these conditions tend not only toward the destruction of the red cells, but likewise to the production of hemoglobinemia (see *Hemoglobinemia*, page 42) and, as a consequence, hemoglobinuria. Potassium chlorate, chromic acid, antifebrin, pyrogallic acid, and CO, when administered in toxic quantities, are capable of effecting essential destructive changes in the hemoglobin, and similar changes are also seen following severe malarial intoxication. As a rule, when the above changes occur in the blood, the red cells are decidedly lessened, but because of the fact that the hemoglobin, though extracted from the red cells, is retained in the serum, this percentage of hemoglobin may not be greatly reduced, yet a decided impoverishment, as a rule, soon follows. Cases have been recorded, however, wherein the hemoglobin suffered decided reduction. The coagulability of the blood may be increased, and the alkalinity moderately lowered. The reports of clinical observations available for criticism are too scanty to warrant deductions.

Purpura Hæmorrhagica.—This condition is doubtless not infrequently of bacteriologic origin (see *Bacteriology of the Blood*, page 114), yet many cases are recorded wherein no evidence of bacteremia existed, which separates the disease into two classes. Purpura developing during the course of an infectious disease shows but slight reduction in the red cells; but in more severe cases absolute diminution may ensue. Microcytes are numerous, nucleated red cells occur, and the color-index is low. Polychromatophilia, leukocytosis, and eosinophilia are of less common occurrence. Ewing noted 56,000 leukocytes per cubic millimeter. Coagulation is feeble, and many writers refer to a reduction in the blood-plates. Other competent observers, however, have not been able to confirm this finding.

The non-infectious type may hematologically resemble pernicious anemia, the differential feature being that in purpura hæmorrhagica there is an absence of megaloblasts; normoblasts are scanty, and microcytes numerous. Leukocytosis does not occur in this form of purpura; but the lymphocytes occupy from 75 to 90 per cent. of the total number of leukocytes. The red cells fall rapidly to 2,000,000, 1,000,000, or less per cubic millimeter. The majority of the erythrocytes are undersized, oval, and in the latter respect resemble those in pernicious anemia.

Scurvy.—The morphologic changes displayed by the blood of scurvy are similar to those observed in ordinary anemia following hemorrhage. The red cells fall to 3,000,000 or less, and fragments of disintegrated cells are to be seen. The hemoglobin index is reduced. Leukocytosis has been observed by a number of investigators, while many have been unable to detect leukocytes beyond the normal maximum limit.

Hemophilia.—In hemophilia decreased coagulation of the blood has been observed, and was found to be the only hematologic change conspicuous in a case studied through the courtesy of Dr. Ernest Laplace at the Medico-Chirurgical Hospital. After the administration of adrenalin chlorid coagulation was found to be more rapid. The fibrin is about normal in amount. Further blood changes are usually the result of hemorrhage, and to arrive at any definite conclusions the blood of hemophilia must needs be studied when the bleeder is apparently in perfect health (see *Blood-plates*, page 100).

Addison's Disease.—In Addison's disease the red cells are commonly diminished to from 2,000,000 to 3,000,000 per cubic millimeter. Microcytosis is conspicuous; the leukocytes are normal or subnormal, and of these, eosinophils occupy the high normal percentage.

Myxedema.—Myxedema when uncomplicated and not subjected to treatment displays a secondary chlorotic anemia, with a mild grade of leukocytosis. The hemoglobin registers 60 to 70 per cent. or less, and the red cells are found to be greatly reduced. The administration of thyroid extract may be followed by appreciable improvement in the hematologic tissue, which may result even in polycythemia.

PARASITIC DISEASES AFFECTING THE BLOOD.

UNCINARIA DUODENALE (ANKYLOSTOMA DUODENALE).

Infection with this parasite usually results in a 'profound anemia, which is characterized by a low percentage of hemoglobin and low color-index; red cells 1,000,000 or less in severe cases, and poikilocytosis well marked. Normoblasts are frequently encountered, and in extreme instances megaloblasts are not uncommon findings. In uncomplicated cases the leukocytes are normal, and eosinophilia commonly pronounced, though by no means a constant feature of the blood of uncinariasis. The blood changes are set forth by Surgeon Bailey K. Ashford, pages 144, 145.

The corpuscles of patients suffering from ankylostomiasis are easily agglutinable. The serum of persons thus infected is more actively hemolytic for the blood of rabbits than is normal blood; but is not hemolytic for either healthy or pathologic bloods of man. Serum from persons infected with the uncinaria is highly toxic for rabbits, inducing hemoglobinuria and often causing death (Romanis).*

STRONGYLOIDES INTESTINALIS.

Strongyloides intestinalis is not infrequently associated with the ankylostoma. Ewing cites Teissier as having reported the discovery of numerous embryos of the anguillula in the blood of intermittent fever. Moderate eosinophilia—10 to 13 per cent.—has been observed. (See p. 419.)

ASCARIS LUMBRICOIDES, OXYURIS VERMICULARIS, TRICHOCEPHALUS DISPAR.

These parasites are capable of producing a severe grade of secondary anemia. The trichocephalus is associated with ankylostomiasis, and its ova have been present in the feces in all cases of ankylostomiasis studied by the author. Fatal anemia may follow infection with any of these parasites, when the red cells may number below 2,000,000 and the hemoglobin 40 per cent. or less. Leukocytosis is seldom observed, but eosinophilia is a common finding in cases of Ascaris lumbricoides. I have repeatedly found the eosinophils between 10 and 18 per cent. in children infected with the oxyuris, but it was impossible to say definitely that this eosinophilia resulted from this parasite alone, since these children were always of lowered vitality, anemic, and, being hospital cases, blood examinations after the institution of treatment were not feasible.

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* "Gazzetta Degli Ospedali e Delle Cliniche," Nov. 27, 1904.

SCHEDULE OF BLOOD EXAMINATION.

Current Number.	Red Cells.	Hemoglobin.	White Cells.	Percentage of Polymorphonuclear Leukocytes.	Percentage of Small Lymphocytes.	Percentage of Large Lymphocytes.	Percentage of Eosinophils.	Normoblasts per Cubic Millimeter.	Megaloblasts per Cubic Millimeter.	Remarks.
1	1,530,116	20	6,800	65	21	9	5	26	13	Poikilocytosis; polychromatophilia; many microcytes and macrocytes.
2	697,776	20	7,960	59	17	15	9	144	46	Poikilocytosis; polychromatophilia; many macrocytes and microcytes.
3	1,533,112	22	2,000	64	22	8	6	8	0	Poikilocytosis; no rouleaux.
4	1,200,000	15	4,200	64	23.4	6	6.6	109	8	Poikilocytosis; polychromatophilia.
5	1 484,440	10	6,000	64	24	8	4	12	0	Poikilocytosis; slight tendency to rouleaux formation; macrocytes and microcytes, latter predominate over the former.
6	2,193 328	23	8,800	65	26	8	1.25	123	35	Poikilocytosis; no tendency to rouleaux formation.
7	1,633,328	17	5,600	60	23	5	12	11	0	Poikilocytosis; many macrocytes and microcytes; slight rouleaux formation; marked chromatophilia.
8	2,064,664	23	4,800	74	17	6	3	28	0	Poikilocytosis; rouleaux formation good.
9	1,271,104	14	7,800	60	17	6	17	30	15	Has elephantiasis Arabum, but I cannot identify the filaria as yet; microcytes predominate over macrocytes; good rouleaux formation.
10	1,600,000	17	1,500	72	20	4	4	6	0	Poikilocytosis.
11	1,800,000	25	4,600	69	22	7	2	9	0	Poikilocytosis.
12	2,266,656	30	7,680	52	26	12	10	0	0	
13	1,268,888	20	6,800	23	17	7	13	0	0	Poikilocytosis; rouleaux formation good.
14	2,440,000	25	11,000	50	10	9	31	0	0	Poikilocytosis; rouleaux formation slight; has abscess of liver.

15	2,353,328	17	12,700	73	17	4	6	0	0	Poikilocytosis; rouleaux formation.
16	2,934,444	..	5,200	72	20	5	3	10	10	Tertian malarial parasites found; rouleaux formation good.
17	2,140,000	23½	18,000	40	12	8	40	36	0	Poikilocytosis; rouleaux formation good. I believe this man to have been suffering from pneumonia at time of examination, although the differential leukocyte count seems strange.
18	3,524,440	55	14,800	88	9	3	⅓	0	0	Only case not presenting ankylostoma. Has tuberculosis.
19	Unknown.	30	9,000	60	16	10	10	125		Poikilocytosis; rouleaux formation good.
20	1,560,000	16	2,400	72	10	5	13	0	0	

TRICHINOSIS.

Infection with the Trichina spiralis usually causes eosinophilia (15 to 50 per cent.) to develop with the onset of the symptoms, and to continue for weeks and months after the acute manifestations have subsided. There appear to be but few records wherein the blood of trichinosis has been studied for eosinophilia after the patient had supposedly recovered. In one case observed the eosinophils were at a high normal limit one year after the onset of the disease. Increase of the eosinophils is accompanied by a reduction in the neutrophil cells, and vice versâ.

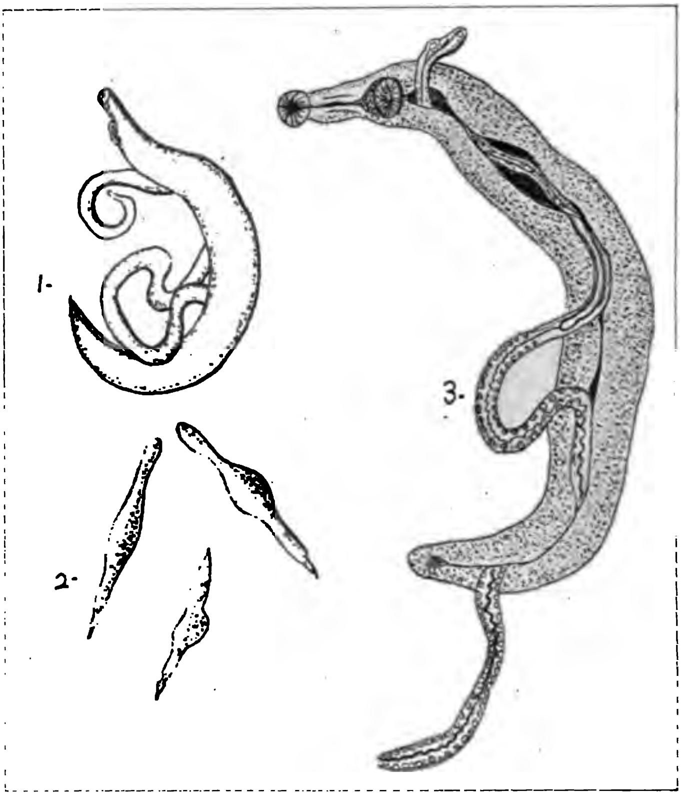

Fig. 48.—1, Bovine blood fluke (Schistosoma bovis), male and female (after Leuckart); 2, ova of bovine fluke (after Sonsino); 3, human blood fluke (Schistosoma hæmatobium), male and female; magnified (after Looss).

Leukocytosis is not an uncommon symptom, yet by no means is it a constant one. Brown found the leukocytes to number

35,700 in one instance. In my own case they never numbered above 7500. The hemoglobin may fall from 75 to 50 per cent., and the red cells at the onset number 4,000,000 or more; but when the infection is severe, the erythrocytes are markedly reduced from the sixth to the tenth week of the disease. Trichinosis cannot be diagnosed upon the blood findings alone, and all importance is attached to the detection of the embryos in the muscle tissues (see *Trichinosis*, page 422).

SCHISTOSOMA (DISTOMA, BILHARZIA) HÆMATOBIUM.

The adult parasites (Fig. 48, 3) inhabit the veins of the portal system, where they are to be found in great numbers. Their ova seldom enter the general circulation, however, but are to be found in the veins of the rectum and of the bladder. Ulceration of the mucous surfaces ensues when the ova (Fig. 48 and Plate 19 A) escape and are found entangled in the blood-clots of the urine or of the feces (see *Parasitic Hematuria*, page 295). Anemia may follow hemorrhage from the bladder, which is apt to be persistent, continuing for a period of several years. Early after infection eosinophilia has been detected.

DIBOTHRIOCEPHALUS LATUS, TÆNIA SOLIUM, AND TÆNIA MEDIOCANELLATA.

A severe secondary anemia may result from infection with any one of these parasites. The bothriocephalus is claimed by foreign authors to excite an anemia resembling closely, if not identical with, pernicious anemia. In the study of a number of persons infected with the beef tape-worm I have always found a rather high-grade secondary anemia. In three of these cases normoblasts were found.

FILARIASIS.

The Filaria sanguinis hominis (Fig. 49) is a nematode worm which inhabits the deeper blood-vessels or lymphatics, and usually discharges its embryos into the circulating blood, yet by no means are embryos found in the blood in all cases of filarial infection. According to Manson's classification, the embryos are most numerous in the blood at certain periods, and he has named the different organisms, with reference to their periodicity: *Filaria diurna*, which occupies the deeper vessels during the night and invades the circulation during the day; *Filaria nocturna*, which courses through the peripheral circulation at night; and the *Filaria per-*

stans and *Demarquaii*, which are present in the circulation both by day and by night.

Method of Detection and Staining.—Special Technic for the Study of the Filaria (see page 74).—In smearing blood upon slides or cover-glasses it is well that films be made rather dense in order to include several organisms upon a single slide. Fix the blood by heat, stain one-half minute with a 2 per cent. solution of eosin in 70 per cent. alcohol, and counterstain with a 2 per cent. solution of methylene-blue for from one-half to two minutes—the operculum will display a light-pink tinge, while the inclosed parasite stains blue. The filaria is readily detected in the stained specimen even by the inexperienced eye, and to those not familiar with the parasite this method of research is commendable In searching for the living embryos in the fresh specimen of blood a two-thirds objective should be employed, under which they appear as minute wriggling serpents. After the parasite is located with a low-power objective it may be studied in detail through a one-sixth or a one-eighth objective.

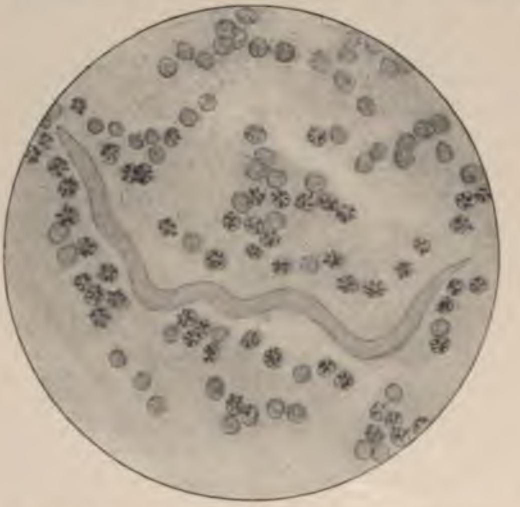

Fig. 49.—Filaria sanguinis hominis. Living filaria in blood from case at Pennsylvania Hospital. Sketch forty-eight hours after blood was taken.

Clinical Significance.—The filaria is an etiologic factor in parasitic elephantiasis and in chyluria. The Filaria perstans has been found repeatedly in the blood of persons suffering from sleeping sickness, but no alliance has been proved to exist between filariasis and sleeping sickness (spinal trypanosomiasis, page 151).

DRACUNCULUS MEDINENSIS (GUINEA-WORM).

Dracontiasis, or guinea-worm disease, like filariasis, is a tropic affection caused by the Dracunculus medinensis; the adult parasite inhabiting the human connective tissue, and belonging to the class of nematodes (Fig. 50, *B*). Its habitat is the western coast of Africa, India, Brazil, and Arabia. According to statistics, a single authentic American case has been reported by Edward Francis,* though there appear records of two additional cases. The adult female worm is cylindric, about 26 inches in length, and one-tenth of an inch in diameter, of a milky color, smooth surfaces, with

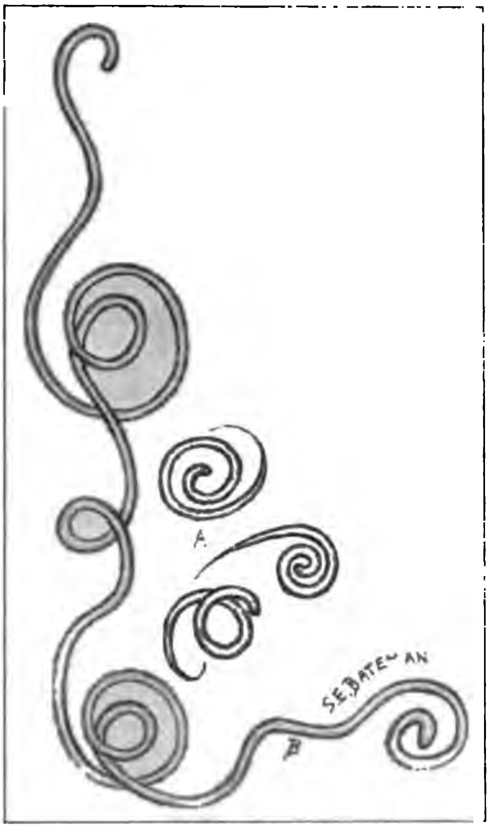

Fig. 50.—*A*, Embryo of guinea-worm (Dracunculus medinensis); *B*, adult female guinea-worm (after Bristow).

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* "Amer. Med.," Oct. 26, 1901, p. 651.

a tapering tail that is abruptly bent near its tip. The head is provided with a triangular mouth surrounded by six papillæ. The uterus extends nearly from the head to the tail, and is filled with embryos. R. H. Charles discovered a worm in the mesentery of a cadaver, and regarded it as the male of the Dracunculus medinensis. The exact method of infection with this parasite remains moot, though the cyclops, a small crustacean, probably serves as an intermediate host.

The Embryo.—The embryo is nearly one-half of an inch in length,—5 to 75 mm. by 0.015 to 0.025 mm. (Manson) (Figs. 50, 51),—its head tapers slightly, and the tail is long and sharply pointed. The alimentary canal is readily distinguishable. The impregnated female makes her way through the intracellular connective tissue for the period of from nine to twelve months, and when fully matured, she burrows her way toward the legs and probably to the feet, and now appears near the surface of the body and emerges from the derma. The uterus ruptures and the embryos are discharged in a whitish fluid. Symptoms of the guinea-worm do not develop until the parasite is fully matured, when the initial feature is the appearance of a vesicle or abscess at the site where it emerges through the skin. This may be preceded for a few days by localized swelling, a feeling of tension, sensitiveness, and redness, and in many instances the worm may be felt beneath the skin. Developing from the abscess is an ulcer which is more or less extensive, and from which the head of the adult parasite may protrude.

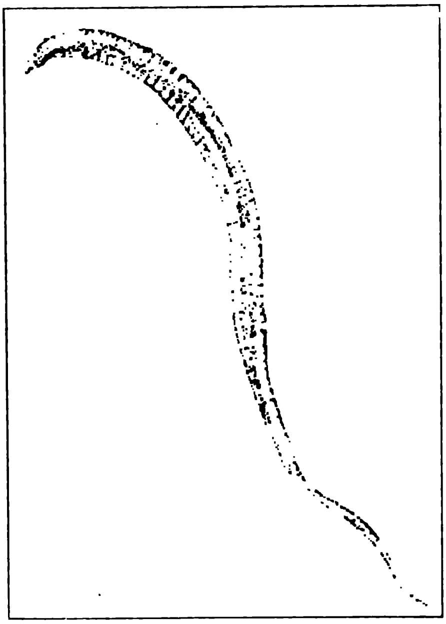

Fig. 51.—Embryo Dracunculus medinensis (guinea-worm), highly magnified (after Francis).

Detection.—The milky discharge collected from the ulcer, when placed upon a slide and studied microscopically, will be found to contain a number of embryos (Fig. 51); and when spread upon a slide, fixed by heat, and stained with carbol-thionin, they may be mounted in Canada balsam. Should the embryo be mounted in Canada balsam without staining it will be impossible to detect the parasite (Francis).

TRYPANOSOMIASIS.

(Surra; Tsetse-fly Disease; Nagana; Mal de Caderas; Dourine; Mal de Coit; Pjudi.)

The trypanosoma is a flagellated hematozoon which has been found in the blood of man along the Gambia River, in Algiers, and a case, the fourth, in which the parasite was detected is reported by Dr. Le Moal as having occurred in the French Congo.* It is not unlikely that the commissions authorized by the various European governments for the investigation of malaria in South African colonies and tropic districts will furnish valuable data regarding the frequency of the trypanosoma in man. Manson† has outlined the clinical pictures of two cases of trypanosomiasis, and in view of his descriptions it seems fair to believe that the disease is more common than would appear on first consideration. The parasite is to be found in the blood from the peripheral circulation during the febrile stage of the disease, but during the afebrile period it is seldom encountered, though they become numerous at each successive recurring pyrexia. From one to six parasites may be counted in a three-fourths of an inch blood-film prepared during the febrile stage. Thus far no relation has been found to exist between the degree of temperature and the number of parasites present in the blood.

The Parasite.—In size, the Trypanosoma hominis varies from 18 to 26 μ in length, and from 2 to 2.5 μ in breadth. In the peripheral blood are to be seen large, roundish bodies 14 to 16 μ in diameter, showing one or more vacuoles which are usually regarded as being a developmental stage of the parasite (Fig. 52). The structure and habit of the Trypanosoma hominis resemble the trypanosomes common to the blood of the lower animals, among which may be given rats, rabbits, cats, dogs, mice, bats, monkeys, horses, mules, cattle, camels, elephants, buffaloes, and sheep. Trypanosoma gambiense (Dutton) is a minute, colorless, translucent, active vermicule, tapering toward its extremities, the anterior of which is provided with a long flagellum—a continuation of an undulating membrane attached for nearly the entire length of one side of the body—which is of alveolar structure (Figs.

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* "Le Caducée," Dec. 20, 1902.

† "Tropical Diseases," third ed., p. 178.

52, 53). That portion of the parasite which may be regarded as the body often shows indistinct minute granules and "very generally a refringent speck, a centrosome, near the posterior end." The trypanosoma is found free in the plasma and has not been seen within the corpuscle. E. J. Moore,* while making daily examinations of the blood of infected persons—natives of West Africa—believes that certain peg-shaped bodies seen in the red cells represent the intracorpuscular parasite. These comma-shaped refractile bodies are also seen in the plasma. Manson has observed parasites displaying evidence of longitudinal division. For demonstration of this parasite in laboratory work the blood of rats will be found to serve adequately this purpose, since they are usually infected with a form of trypanosoma. Inoculation of susceptible animals with the blood of infected rats is capable of producing the disease.

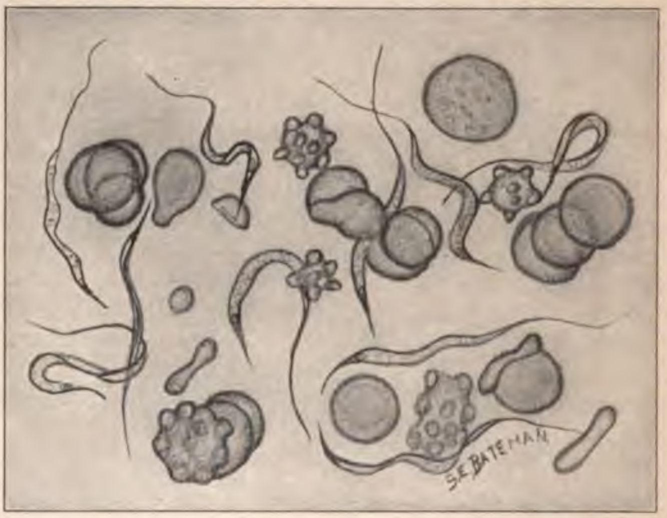

Fig. 52.—Trypanosoma from blood of gray rat; also nodular cells, probably preparing for division.

The **technic** for the recognition of the Trypanosoma hominis differs in no way from that given for the detection of the malarial parasite in the fresh blood (page 157). It is seen best with an oil-immersion objective, and is indistinguishable unless magnified 300 diameters or more. F. G. Novoy has cultivated the trypanosoma of rats on a medium composed of rabbit's blood-serum and agar. Growth occurs in the condensation liquid, but not upon the surface of the medium.†

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* "Lancet," Oct. 1, 1904.

† "Jour. Amer. Med. Ass.," June, 1902, p. 1653.

Staining.—The Trypanosoma hominis is stained by the ordinary anilin dyes. I prefer the following method: Smear the blood rather thinly and fix by heat, when stain for from one-half to one minute with solution of 1 per cent. eosin in 70 per cent. alcohol, wash in water, and stain for fifteen seconds with a 2 per cent. aqueous solution of methylene-blue, wash in water, and again stain for five seconds with eosin, wash, and mount

Sleeping Sickness.—Aldo Castellani detected the trypanosoma in the cerebrospinal fluid obtained by lumbar puncture from a case of sleeping sickness in 1892, and has since found this parasite in the cerebrospinal fluid of 34 additional cases,[*] and in the blood in one instance (see *Filaria perstans*, page 148).

Bruce obtained spinal fluid by lumbar puncture from 38 cases of sleeping sickness and detected the trypanosoma in the fluid from every case. The parasite was also found in the blood of 12 out of 30 cases thus studied. Chatterjee[†] found a trypanosome in the proboscis of a mosquito.

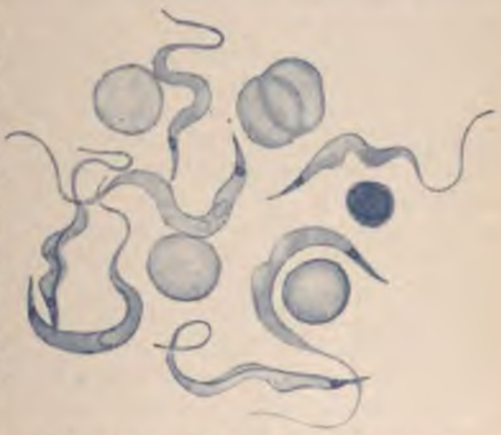

Fig. 53.—Trypanosoma from blood of gray rat; stained with a 2 per cent. aqueous solution of methylene-blue.

Animals.—Musgrave and Williams estimate the loss by death of horses in the Philippine Islands from "surra" (trypanosoma infection) at $2,000,000 a year.[‡] The symptomatology of animals has suggested to me that possibly certain cases of the so-called "non-parasitic elephantiasis" were in reality trypanosomiasis.

MALARIA.

ZOOLOGIC AFFINITIES.

Entering upon a study of the malarial micro-organism (hematozoon of Laveran) it appears warrantable first to direct the student's attention to the zoologic affinities of this animal parasite, and to the resemblances known to exist between this and other allied blood parasites.

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[*] "Lancet," June 20, 1903.

[†] "Indian Medical Gazette" Oct., 1901.

[‡] Dept. of the Interior, U. S. A., 1903.

Zoologically, the hematozoon of Laveran belongs to the class of sporozoa and is somewhat closely allied to the coccidia (Plate 24, page 388). On account of certain peculiarities common to the malarial parasite and to other parasites of the lower animals it has been classed with these parasites in the special orders, which have been appropriately named hemameba, hemamebida, hemosporidia, etc. The malarial parasite resembles the coccidia in that a portion of its life is spent within the body-cell of the host, selecting especially the red blood-corpuscle for this portion of its life-cycle.

Up to the present time the malarial parasite is not known to affect other vertebrates than man; but parasites closely allied to the malarial micro-organism invade the blood-corpuscles of vertebrates—viz., dog, bat, monkey, horse, birds, and cattle. Little is known regarding these parasites except of the Pirosoma bigeminum (Fig. 55), the cause of Texas fever in cattle, which has been extensively studied by Theobold Smith, of America.

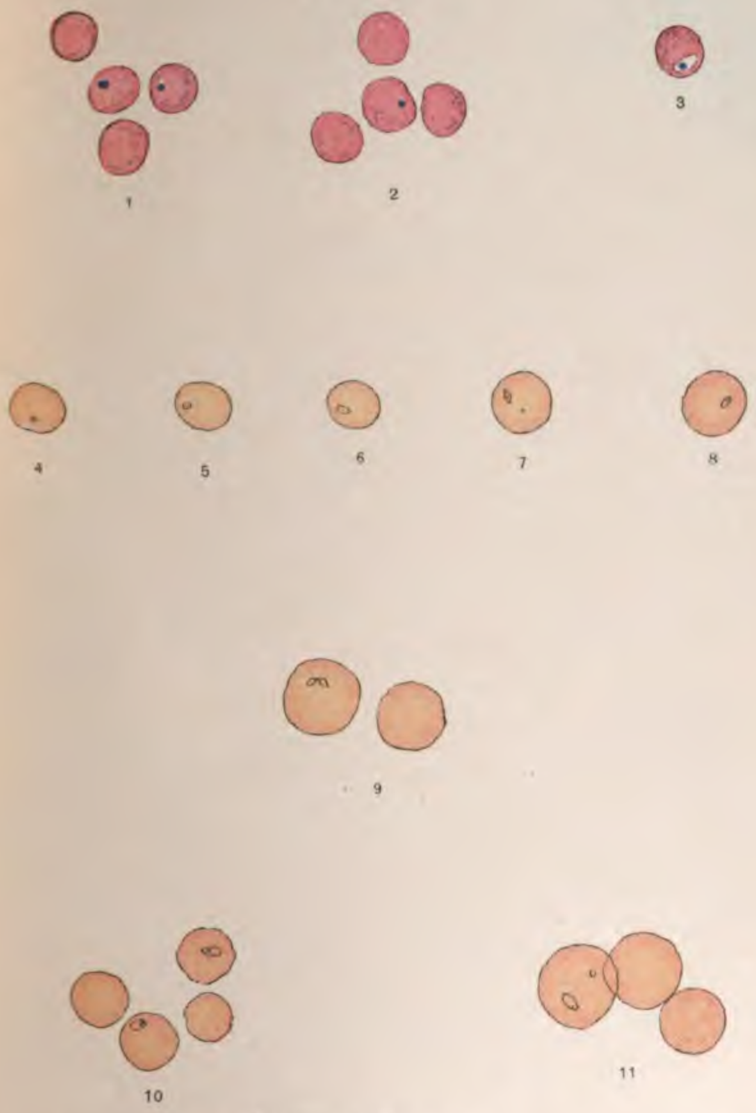
The animal parasites invading the blood plasma and the corpuscles of birds and reptiles, such as halteridium, proteosoma, and drepanidium, have of recent years received careful study. The vast amount of light thrown upon the true life-cycle of the malarial parasite through the comparative study of the parasites of other vertebrates is evidenced by the fact that MacCallum discovered the method of sexual fertilization of the malarial organisms while studying the blood of birds infected with the halteridium (Fig. 54). According to Laveran, these animal parasites (hæmocytozoa) were arranged under three subclasses.

1. HÆMAMEBA.—A genus characterized by the development of pigment and by an asexual (endogenous) and a sexual (exogenous) life-cycle (Fig. 66). The sexual or exogenous cycle is completed through the agency of the mosquito. This genus includes the malarial parasite.

NAMES.	HOSTS.
H. subtertiana H. tertiana H. quartana	The malarial parasites of man, the sexual phase being evolved in mosquitos of the genus anopheles.
H. (Proteosoma) relicta	Birds; sexual phase in mosquitos of the genus culex.
H. (Halteridium) Danilewskyi	Birds; sexual phase probably the mosquito.
H. Kochi	Several species of monkey; sexual phase not determined.
H. melaniphera	Bat (Miniopterus Schreibersii); sexual phase not determined.
H. Metchnikovi	Trionyx indicus; sexual phase not determined.

2. PIROPLASMA.—The species of this genus are devoid of pigment and have an asexual (endogenous) reproduction, probably effected by binary division, and a sexual phase of develop-

PLATE 10.



Piroplasma Hominis.

1, Small form of the parasite found in one field; 2, the same, another field; 3, showing parasite with central stained spot surrounded by a vacuole. (Figs. 1–3 stained with Wright's stain, followed by Löffler's blue. × 750.)

4, 5, Small form of the parasite (× 665); 6, large form (× 665); 7 and 8, single form of the parasite (× 1000); 9, double form of the parasite (× 1000); 10, one field showing two infested corpuscles (× 665); 11, one field showing a corpuscle with a large and small parasite (× 1000) (Figs. 4–11 from fresh blood) (Anderson).

ment which takes place in various species of tick. In human piroplasmosis the parasites are found in the peripheral blood, in that from the viscera, bone marrow, and the exudate from skin and intestinal ulcers. In the red cells they are usually situated near the periphery. They are oval, bacillary, or pear-shaped, and measure about one μ in diameter. Organisms are not numerous in the capillary circulation (Plate 10).

NAMES.	HOSTS.
P. bigeminum	Bovines; transmitted by the cattle-tick (Boöphilus bovis).
P. canis	Dogs.
P. ovis	Sheep.
P. equi	Horse.
P. hominis	Man.
P. hominis of mountain spotted fever	Transmitted by the tick (Dermacentor reticulatus).—Not confirmed by more recent researches of Chas. Wardell Stiles.

3. HÆMOGREGARINA.—The species of this genus infest the blood of cold-blooded vertebrates, viz., frogs, lizards, snakes, etc. They may assume the form of vermicules, and may be found either within the red corpuscle or free, swimming in the plasma (Fig. 56). The life-cycle of this genus is unknown.

NAMES.	HOSTS.
H. (Drepanidium) ranarum	Frog (Rana esculenta).
H. splendens	
H. magna	
H. lacertarum	Lizard (Lacerta muralis).

4. PROTEOSOMA AND HALTERIDIUM.—These pigmented parasites are known to invade the blood-corpuscles of birds. The student should compare figure 54 with plate 11 in order to appreciate fully the resemblance between these micro-organisms and that of malaria. Both these bird parasites have an asexual (endogenous) cycle of development. The proteosoma has its sexual development in the mosquito (culex), and the halteridium is doubtless transmitted in some allied manner.

The study of these parasites is readily accomplished by studying the blood of sparrows; the halteridium is common in the blood of doves, pigeons, and crows. Cleanse the inferior surface of the bird's toe, make a puncture near the tip, and continue the study as for malarial parasites (page 157).

5. PIROSOMA BIGEMINUM.—This animal parasite is concerned in the production of Texas cattle fever, a disease most destructive to the lives of the bovine family in the southern United States, Europe, South Africa, and Australia. The Pirosoma bigeminum (Fig. 55) is devoid of pigment and is to be found in the red blood-cell. Two parasites may occupy a corpuscle. It remained for Theobold Smith to discover that the pirosoma was

transmitted from one vertebrate host to another by the cattle-tick (Boöphilus bovis). In feeding upon the blood of infected cattle the tick takes in the parasite and in some unknown manner infects her ova. Young ticks hatched from this infected mother tick's eggs infect healthy cattle through their bites, a circumstance

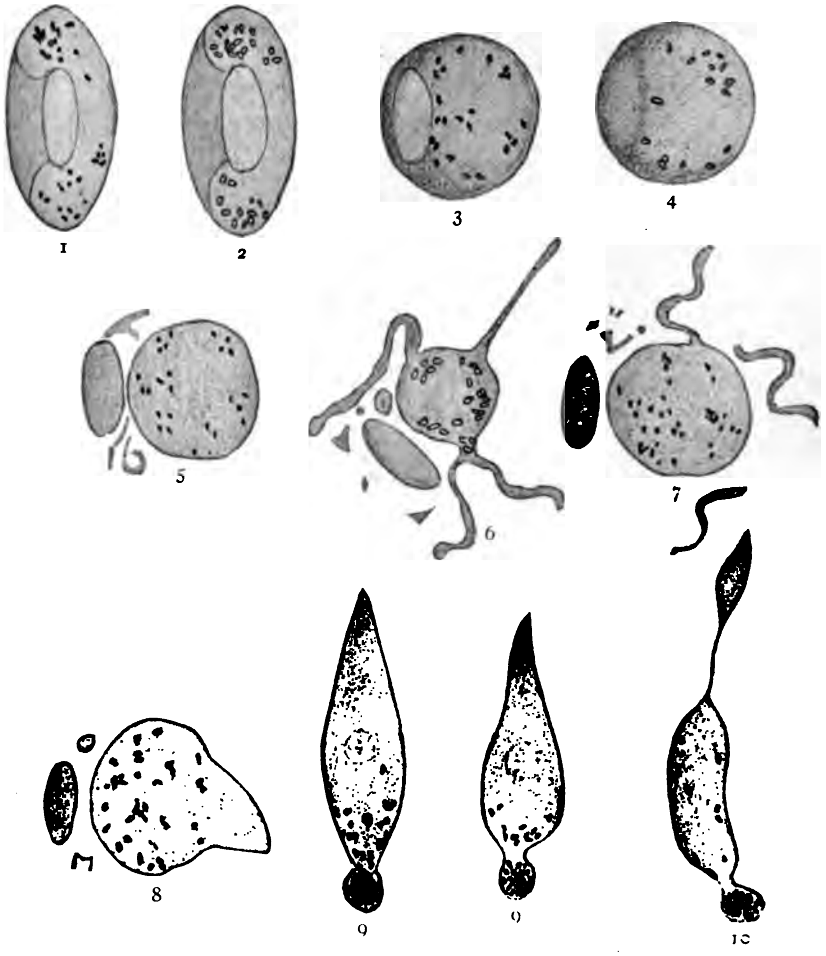


Fig. 54.—Halteridium form of the parasite of birds: 1, Adult granular or female form; 2, adult hyaline or male form; 3, adult female preparing for extrusion from the corpuscle; 4, adult male preparing for extrusion from corpuscle; 5, adult female extruded and lying beside the nucleus of a corpuscle; 6, flagellate form; 7, process of fertilization; 8, first stage in formation of motile form; 9, fully developed motile form or vermiculus; 10, fully developed motile form in process of disintegration (from original illustrations by W. G. MacCallum, "Jour. Exp. Med.," 1898, p. 136).

somewhat analogous to the transmission of malaria by the bite of the mosquito.

6. **Drepanidium.**—This is a non-pigmented blood parasite of frogs (Fig. 56), which is found both as an endocorpuscular, non-motile body and also free in the plasma. The free parasite may display movement.

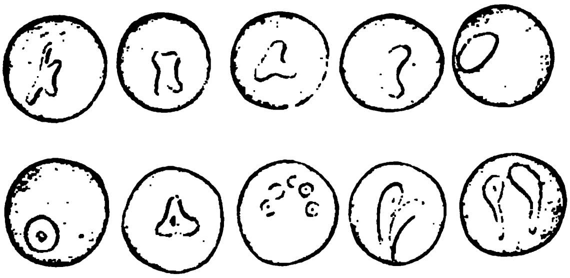


Fig. 55.—Pirosoma bigeminum in bovine blood (after Celli).

THE MALARIAL PARASITE.

Method of Examination.—Collect the blood as described on page 33 from the tip of the finger or the lobe of the ear. The drop should not be too large, and this can be estimated only by sufficient practice to enable one to place beneath the cover-glass just enough blood so that when spread between the cover-glass and slide a film of such thickness results as to hold the cells in close proximity, not touching, and equally disseminated throughout the field. The malarial parasite, a small hematozoon situated within the red corpuscle, may be hyaline, granular, or pigmented, and its contour is circular, oval, or irregular. After it has destroyed the hemoglobin of the red cell the parasite becomes swollen, pale, and granules of pigment displaying Brownian movement are seen within its body, which at times occupies

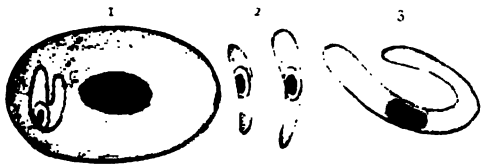


Fig. 56.—Drepanidium ranarum: 1, Parasite within the red cell; 2, 3, extracellular motile (forms after Celli).

nearly the entire red cell. The various forms of malarial hematozoa are best shown by the accompanying illustrations (Plates 11–13).

Staining of the malarial parasite is not essentially different from that of staining ordinary blood smears. The films are spread in the same manner, dried in the air, and later may be fixed in any of the methods described for fixation of the blood (page 74). Futcher recommends immersing the dried films in a 0.25 per cent. solution of formalin in 95 per cent. alcohol for one minute, after which rinse in water. However the fixing may be accomplished, the malarial parasite is well stained by a carbolated solution of thionin, and by this solution it appears as a conspicuous, reddish-violet body.

Carbol-thionin.—Carbol-thionin is prepared as follows: Saturated solution of thionin in 50 per cent. alcohol, 10 c.c.; aqueous solution of phenol (1–40), 100 c.c.; when mixed, keep in a colored bottle. This stain is of special service in the staining of ring-shaped bodies found in estivo-autumnal fever, and which do not stain well with eosin and methylene-blue. Jenner's stain is also invaluable for the detection of the malarial parasite (see *Jenner's Stain*, page 78).

Eosin and Methylene-blue.—Eosin and methylene-blue will also be found capable of staining blood and malarial parasites in a manner which will render the latter conspicuous. Plehn suggested the following stain, which has been rather generally employed:

Concentrated aqueous solution methylene-blue	60 c.c.
Solution of eosin in 75 per cent. alcohol (0.5 per cent.)	20 "
Distilled water	40 "
Aqueous solution of sodium hydrate (20 per cent.)	12 drops.

After mixing, the films are stained for five to six minutes, washed in water, dried, and mounted. By Plehn's method the red corpuscles are stained pink (eosin), while all nuclei and the malarial parasites stain blue.

Wright's Method.—At the suggestion of Dr. Thayer, of Johns Hopkins University, the late Dr. Jesse W. Lazear, in studying the structure of the malarial parasite when stained according to Nocht's method, gives the accompanying illustrations (Plate 13). Wright's method is but a practical adaptation of the method of Nocht; and while there are several apparently wide differences in the technic of these methods, they have been found to give similar results.

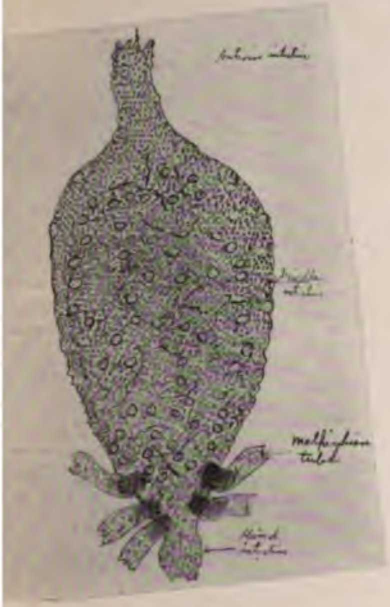
Reference to Wright's method of staining (page 78) and to

Description of Plate 10 A.

Zygotes of Bird Malaria and Human Malaria: Figs. 1–3, zygotes of bird malaria (proteosoma, Labbé) about two or three days old, developing in middle intestine (stomach) of mosquito (Culex). The specimen of infected mosquito was obtained by Dr. Ronald Ross in Calcutta, India, July 12, 1898, and through courtesy was loaned to Dr. Albert Woldert, who made the drawings. The three figures are from the same specimen and represent different degrees of magnification. Fig. 1, middle intestine (stomach) of mosquito, showing zygotes 10 μ in diameter (No. 3 oc., No. 3 lens) (Leitz). Fig. 2, same as Fig. 1, showing ten zygotes of proteosoma about 10 μ in size in one field, $\frac{1}{12}$ oil immer. lens, No. 3 oc. (Leitz). Fig. 3, the zygotes Nos. 1–3 shown in Fig. 2, greatly magnified, and diagrammatic, showing yellowish hyaline capsule and yellowish hyaline, oval-shaped masses (fatty granules?) and vacuoles. Protoplasm has a reticulated appearance and contains reddish pigment granules.

Figs. 4–8, zygotes of human malaria, four days old, developing in middle intestine (stomach) of mosquito (Anopheles quadrimaculata or claviger), obtained by Dr. Albert Woldert, Nov. 5, 1900 (second successful result in America). Figs. 4–8 are from the same specimen and represent different degrees of magnification of zygotes derived from estivo-autumnal parasite (case of estivo-autumnal fever occurring at the Pennsylvania Hospital, service of the late Dr. F. A. Packard).

PLATE 10 A.



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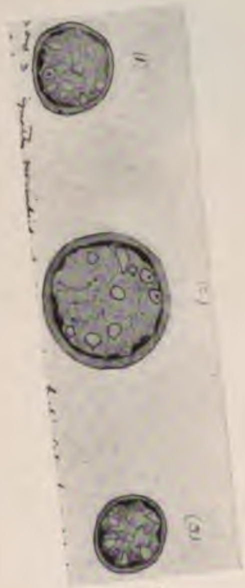

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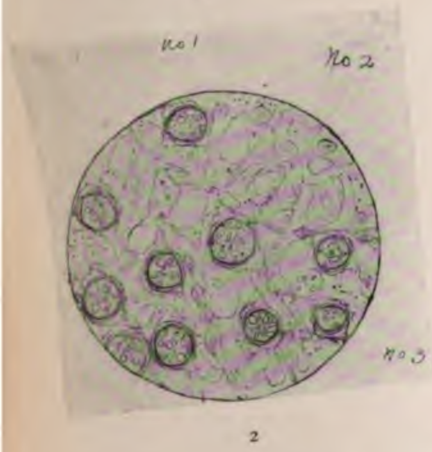

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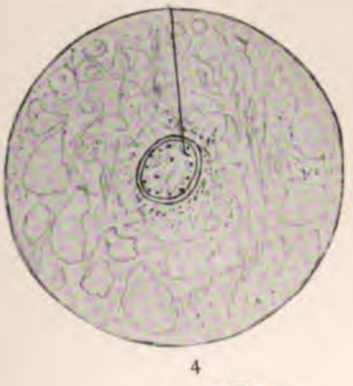

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plate 13, with a careful study of its accompanying key, will be found to direct even the inexperienced.

Hyaline Bodies.—Hyaline or non-pigmented intracellular bodies represent the earlier stages in the development of the malarial parasite, and are to be found in the blood of all forms of malarial infection. They are especially abundant after the paroxysm, and often resemble vacuoles, ameboid movements being their characteristic feature. This movement is most pronounced in tertian fever when the outline of the organism changes with great rapidity. This same change is to be detected in the parasites of irregular estivo-autumnal fever, but, as a rule, the estivo-autumnal parasite assumes a ring-like appearance, and seldom throws out pseudopodia. The number of non-pigmented intracellular hematozoa is at times very remarkable, and in a case originating in Panama and observed at the Philadelphia Hospital every microscopic field of the fresh blood showed several of these organisms.

Pigmented Bodies.—The pigmented intracellular organisms are doubtless a later stage in the development of the parasite, and are likewise encountered in all forms of malarial fever. There does not exist, however, that uniformity in the different forms of malarial infections that is shown in the hyaline bodies (Plate 11); *e. g.*, minute reddish-brown granules often appear in the bodies of the tertian organism soon after the paroxysm, and these increase in number, while their host, the red corpuscle, becomes progressively paler until eventually only an indefinite shell or ring of the once deeply colored erythrocyte remains. It is not uncommon to see aggregations of these granules resembling bacilli, all pigment displaying active movement. The parasite continues to enlarge in its development during the above steps, and its body is now hyaline and may be seen to undergo ameboid changes, but these movements are sluggish. The pigment-granules often appear to be distributed in small aggregations through the body of the red corpuscles, lending the impression that more than one organism has invaded the erythrocyte, but through close observation these isolated patches of granules are found to be within a single organism. Within less than forty-eight hours this malarial parasite will be found to occupy the entire red corpuscle, which is greatly swollen. The ameboid movements now become less and less, though the pigment-granules are often rather active, tending to congregate around the periphery of the cell.

Quartan Fever.—Just following the paroxysm, pigmented intracellular bodies appear in the blood of quartan fever, but here the individual granules display variation in size, and are decidedly darker than are those of the tertian type (Plate 11).

In striking contrast to those of tertian fever they are but slightly motile, often quiescent, and usually accumulate along the periphery of the parasite. Slight ameboid movements are often discernible, but these lessen rapidly and cease in from sixty-four to seventy-two hours, following which the parasite assumes a rounded or oval shape, and occupies but a portion of the red cell. Another peculiarity of this form of malarial fever is that the erythrocytes are not markedly decolorized, but, on the contrary, their normal color may be intensified or they may display a greenish or brassy tinge. The erythrocyte in which the parasite has developed is below the normal size, and upon staining, the organism is found to be surrounded by a variable (usually narrow) zone of cell protoplasm.

Estivo-autumnal Fever.—Pigmented intracellular bodies are likewise found in the blood of estivo-autumnal fever (Plate, 12) but differ decidedly from the tertian or quartan parasites previously described. Pigment-granules appear after the paroxysm, are always scanty, at times only one or two rather dark granules are to be detected, and these are usually located near the periphery of the cell and displaying but slight, if any, movement. This parasite occupies about one-fourth to one-third of the body of the red corpuscle, but does not decolorize the cell, which often exhibits a brassy tint.

The corpuscle may be irregular, shrunken, spiculated, or crenated. Early during the paroxysm the pigment-granules may be congregated at the center of the parasite, and according to certain observers, among whom Thayer, of Baltimore, deserves mention, this peculiar arrangement is to be observed in tertian and quartan parasites during the stage of segmentation.

Segmenting Bodies.—Both the tertian and quartan parasites may be studied under the microscope during the process of segmentation in blood collected just prior to or during the paroxysm. In tertian fever corpuscles will be seen where the hemoglobin has been almost entirely destroyed, as is shown by their pallor. The parasite becomes more and more granular in appearance and the pigment-granules, which at first exhibit movements, become quiescent and enlarged, displaying a tendency to congregate near the center of the organism, where they form in a round mass. While the pigment-granules are being deposited in the center of the cell segmentation of the surrounding granular protoplasm may be observed (Plate 11).

Beginning at the periphery, delicate striations are seen to extend gradually toward the central pigment mass, these striations dividing the protoplasm into a number of rather oval bodies

DESCRIPTION OF PLATES 11 AND 12.[1]

The drawings were made with the assistance of the camera lucida from specimens of fresh blood. A Winckel microscope, objective 1/12 (oil immersion), ocular 4, was used. Figures 4, 13, 23, 24, and 42 of Plate 11 were drawn from fresh blood, without the camera lucida.

PLATE 11.

THE PARASITE OF TERTIAN FEVER.

1.—Normal red corpuscle.

2, 3, 4.—Young hyaline forms. In 4, a corpuscle contains three distinct parasites.

5, 21.—Beginning of pigmentation. The parasite was observed to form a true ring by the confluence of two pseudopodia. During observation the body burst from the corpuscle, which became decolorized and disappeared from view. The parasite became, almost immediately, deformed and motionless, as shown in Fig. 21.

6, 7, 8.—Partly developed pigmented forms.

9.—Full-grown body.

10-14.—Segmenting bodies.

15.—Form simulating a segmenting body. The significance of these forms, several of which have been observed, was not clear to Drs. Thayer and Hewetson, who had never met with similar bodies in stained specimens so as to be able to study the structure of the individual segments.

16, 17.—Precocious segmentation.

18, 19, 20.—Large swollen and fragmenting extracellular bodies.

22.—Flagellate body.

23, 24.—Vacuolization.

THE PARASITE OF QUARTAN FEVER.

25.—Normal red corpuscle.

26.—Young hyaline form.

27-34.—Gradual development of the intracorpuscular bodies.

35.—Full-grown body. The substance of the red corpuscle is no more visible in the fresh specimen.

36-39.—Segmenting bodies.

40.—Large swollen extracellular form.

41.—Flagellate body.

42.—Vacuolization.

PLATE 12.

THE PARASITE OF ÆSTIVO-AUTUMNAL FEVER.

1, 2.—Small refractive ring-like bodies.

3-6.—Larger disk-like and ameboid forms.

7.—Ring-like body with a few pigment-granules in a brassy, shrunken corpuscle.

8, 9, 10, 12.—Similar pigmented bodies.

11.—Ameboid body with pigment.

13.—Body with a central clump of pigment in a corpuscle, showing a retraction of the hemoglobin-containing substance about the parasite.

14-20.—Larger bodies with central pigment clumps or blocks.

21-24.—Segmenting bodies from the spleen. Figs. 21-23 represent one body where the entire process of segmentation was observed. The segments, eighteen in number, were accurately counted before separation, as in Fig. 23. The sudden separation of the segments, occurring as though some retaining membrane were ruptured, was observed.

25-33.—Crescents and ovoid bodies. Figs. 30 and 31 represent one body, which was seen to extrude slowly, and later to withdraw, two rounded protrusions.

34, 35.—Round bodies.

36.—"Gemmation," fragmentation.

37.—Vacuolization of a crescent.

38-40.—Flagellation. The figures represent one organism. The blood was taken from the ear at 4.15 p. m.; at 4.17 the body was as represented in Fig. 38. At 4.27 the flagella appeared; at 4.33 two of the flagella had already broken away from the mother body.

41-45.—Phagocytosis. Traced with the camera lucida.

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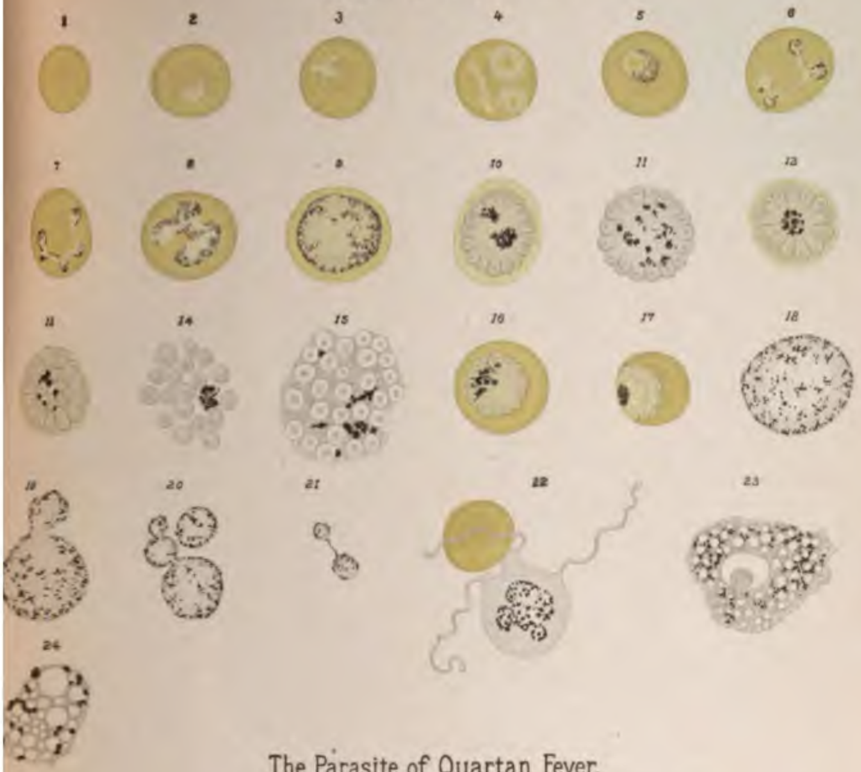
[1] These illustrations are reproduced by permission from the article by Drs. Thayer and Hewetson in *The Johns Hopkins Hospital Reports*, vol. v., 1896.

PLATE II.

The Parasite of Tertian Fever.



The Parasite of Quartan Fever.

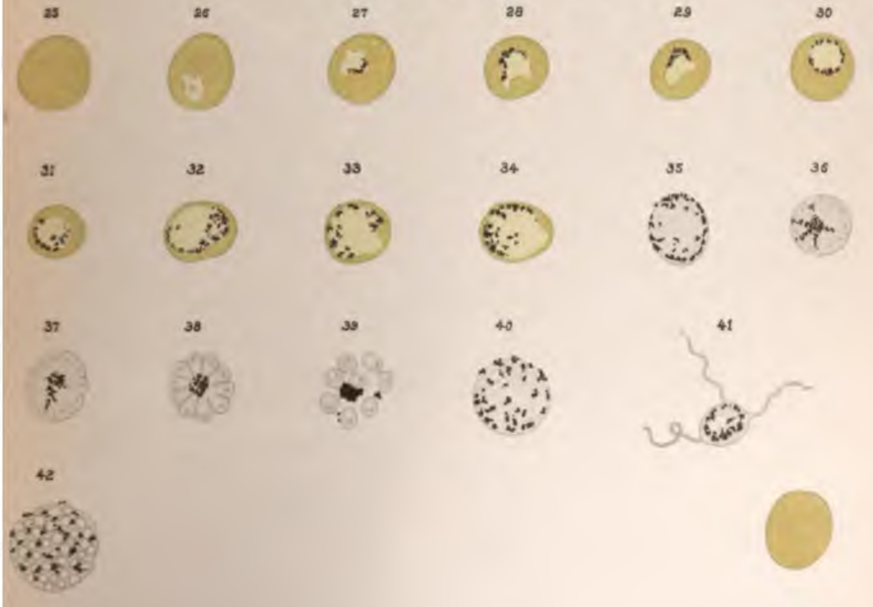
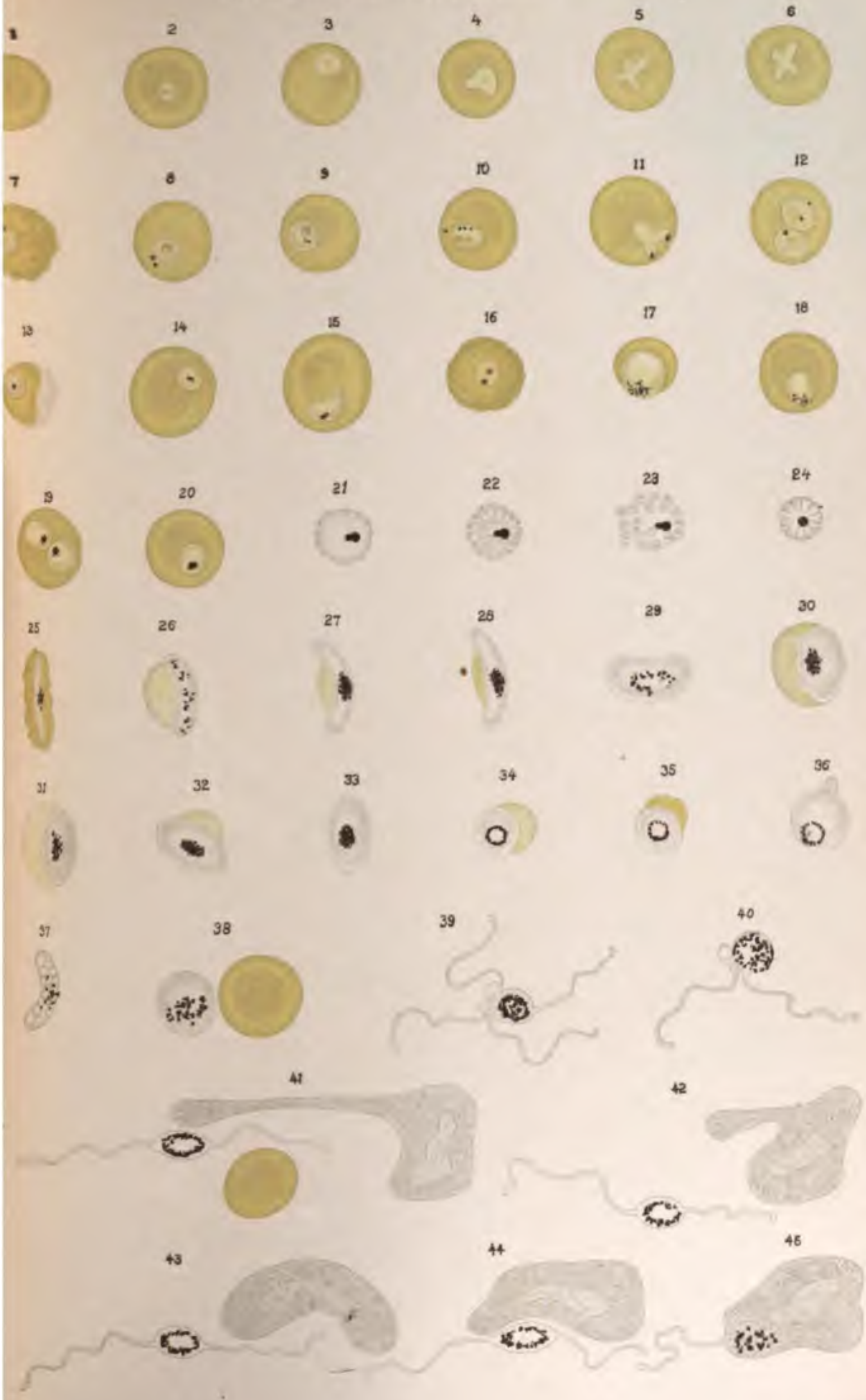


PLATE 12.

The Parasite of Aestivo Autumnal Fever.



(Plate 11). Later the arrangement of these bodies is lost and they are found irregularly distributed through the interior of the parasite. The next step appears to be the absorption or rupturing of the operculum, after which these bodies (sporules) are found disseminated through the plasma. The tertian parasite develops from 15 to 20 sporules, each sporule displaying a minute, faintly granular, dark spot at its interior—a probable nucleus. After the sporules are liberated, they may be seen moving about actively in the plasma, and later they become quiescent. At times segmentation takes place before the pigment-granules collect at the center of the organism. Sporulation, however, is directly associated with the occurrence of the chill, and is regarded as the asexual method for the reproduction of the parasite (Fig. 69).

It is reasonable to suppose that the sporules, after liberation in the plasma, invade healthy erythrocytes, and there develop to the stage of sporulation, thus repeating their asexual or endogenous cycle.

Quartan Parasite.—In quartan fever the parasite undergoes segmentation in a slightly different manner from that described in tertian fever. The pigment-granules are arranged about the periphery of the organism, and as they near the stage of maturity these granules assume a stellate form extending from the periphery to the center, where a dense aggregation takes place. The protoplasm becomes finely granular; when true segmentation begins and continues as in the tertian parasite. The number of segments or sporules are usually from six to twelve, and the segmenting body is always considerably smaller than is that of tertian fever, and its sporules are commonly arranged to form perfect rosets (Plate 11).

Estivo-autumnal Parasite.—Segmenting bodies are not commonly encountered in the blood of estivo-autumnal fever, and it has been suggested that the asexual reproduction occurs in the viscera. When segmentation is to be seen, however, the segmenting bodies are much smaller than are either those of the tertian or quartan fevers, and all evidences of the red cells are destroyed. Crescentic, ovoid, and spheric bodies are always observed in the blood of estivo-autumnal fever after the infection has existed for a period of one or more weeks (Plate 12). For a long period of time there has existed considerable question as to whether or not these bodies represented a definite stage in the life-history of the more common forms of malarial organisms. The weight of opinion, however, seems to favor the fact that crescentic forms are derived from pigmented intracellular forms.

Crescentic Bodies.—Crescentic bodies are rarely to be found within the erythrocytes. The typical crescent is a highly refractile body from 7 to 9 μ in length and 2 μ in breadth, exceeding the red corpuscle in size. Its extremities are rounded, and united with a delicately curved line which expands the concave border of the crescent, and occasionally this marginal, hood-like projection is seen extending along the convex border (Plate 12). Fine granules and rods of pigment are seen within the crescent, commonly collected about the center of its body, but occasionally aggregations of pigment are discernible in one of the horns. The pigment-granules are usually motionless, yet slight movements may be seen where granules occupy one extremity of the crescent.

Oval Bodies.—Both the oval and spheric bodies are apt to be smaller than are crescents, but exhibit similar features, and are often provided with a small hood-like questionable remnant of their former host, the red cell. Either oval or spheric bodies may be seen within the red cells (Plates 11, 12, 13).

Extracellular Pigmented Bodies.—Certain of the extracellular bodies found in both tertian and quartan fevers do not undergo segmentation after they have reached maturity, but, on the other hand, leave their host, the red cell, and appear free in the blood (extracellular pigmented bodies). In their new environment they develop rapidly, and in the tertian form may attain the size of a polynuclear leukocyte. The pigment-granules display more active movements than are to be observed under other conditions; the parasite becomes distorted and its outline indistinct. After a time the movement of the granules gradually lessens and finally ceases; while the body of the parasite becomes more and more distorted, probably resulting from its death.

Again, certain of these organisms may show a gradual fragmentation, and small particles of their pigment are seen to be separated from the general pigment mass. In this manner a parasite may be seen to divide into several smaller bodies (four or five); the movements of the pigment persist in the smaller subdivisions, but later these granules become quiescent, the body of the cell grows less distinct, and death ensues. At times, after the pigment-granules have become motionless the cell may display vacuoles (degeneration).

Flagella.—Generally speaking, the flagella of quartan fever are slightly smaller than are those of the tertian form (Plate 11), and Craig believes that there occur in the blood of cases of tertian and of estivo-autumnal fevers two varieties of flagellated organisms.

PLATE 13.

STRUCTURE OF THE MALARIAL PARASITE.

A1–11. Tertian parasite; asexual generation: 1–9 correspond with the development of the organism, showing chromatin divided more and more and progressive increase in the pigment; 10, presegmenting parasite, showing multiple nuclei of the future segments; 10, segmenting organism.

B 1, 2. Tertian parasite; gametes.

C 1–5. Quartan parasite; asexual generation, showing gradation of organism as evidenced by age: 3, 4, maturer forms; 5, presegmenting form of the parasite. Note especially that the red cell is not enlarged as the result of quartan infection, and that the pigment is much distributed.

D1–4. Estivo-autumnal parasite; asexual generation: 1, 2, 3, early ring forms; 4, mature parasite.

E1, 2, 3. Crescentic bodies: Note staining of hood-like portion of 3, which expands the concavity of the organism.

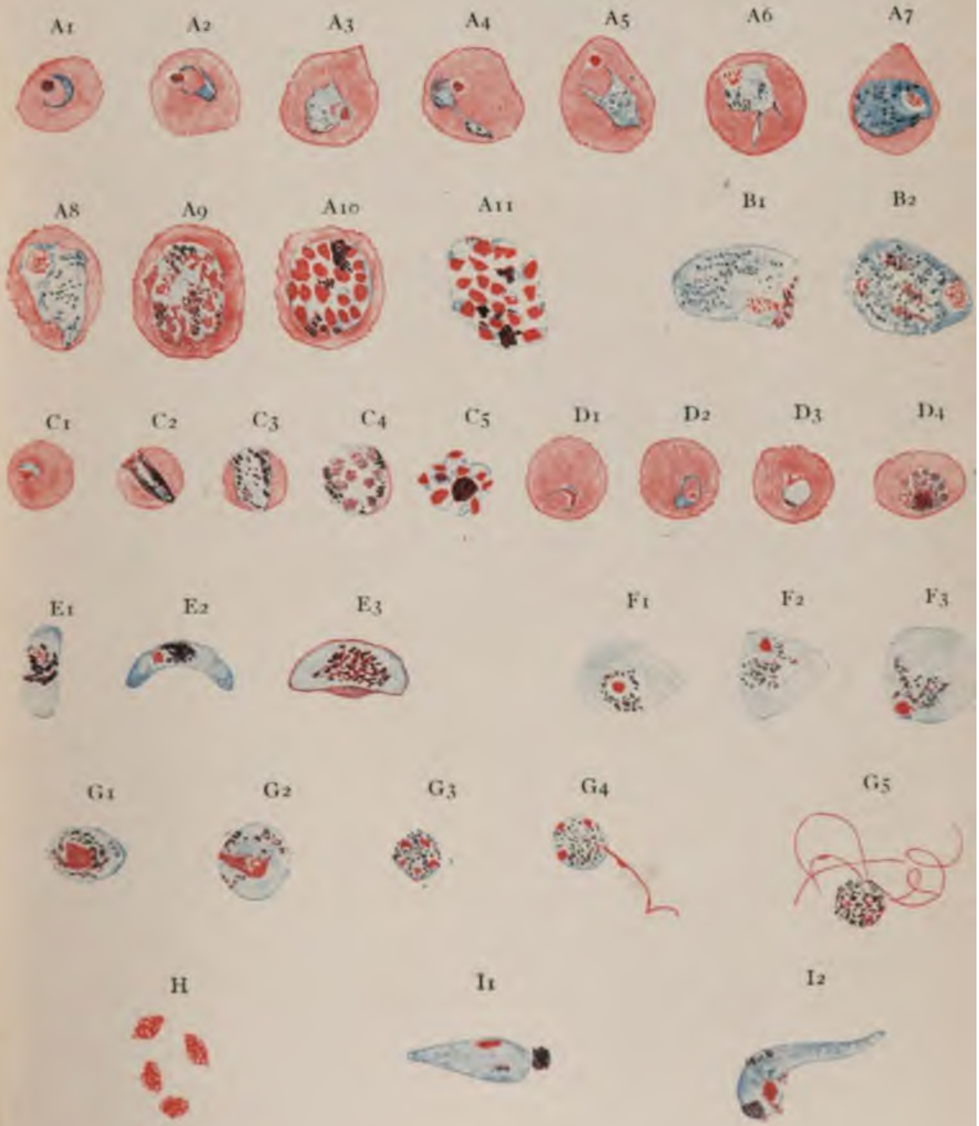
F1–5. Estivo-autumnal parasite; macrogametes (ovoid bodies).

G1–5. Estivo-autumnal parasite; microgametocytes: 4, 5, Show stained flagella.

H. Blood-plates. These are often found resting upon the red cell, and are to be distinguished from malarial infection of such cells.

I1, 2. Pseudovermiculus laverania danilewskyi from crow's blood. (See *Halteridium*, page 155.) (Lazear in Johns Hopkins Hospital Reports, vol. x, Nos. 1 and 2.)

PLATE 13.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C4
C5
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D2
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D4
E1
E2
E3
F1
F2
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G1
G2
G3
G4
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H
I1
I2

Method of Developing Flagella.—Ordinarily flagella make their appearance in from fifteen to twenty minutes after the blood has been withdrawn; rarely I have seen them develop sooner (three to ten minutes). The blood is withdrawn from the finger or ear (see page 33), and after removing the first few drops that collect at the site of puncture a drop is allowed to remain exposed to the air for a few seconds. Breathe upon the surface of the slide until it shows evidence of moisture, when bring the moistened surface of the slide in contact with the drop of blood; add immediately a cover-glass to that portion of the blood clinging to the surface of the slide, and the specimen is ready for microscopic study. Flagellation is hastened by the presence of the moisture supplied from breathing on the slide. The bodies from which flagella develop (tertian fever) are large and may show their pigment either in active movements and disseminated throughout the protoplasm (Fig. 57, *A*, *B*), or the same pigment may be seen but a few minutes later occupying small blotches in the protoplasm, and practically motionless.

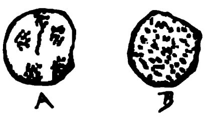

Fig. 57.—Large body of tertian fever, pigment in blocks (*A*); *B*, pigment evenly disseminated (Craig).

Development of Flagella.—Certain of the extracellular organisms contain pigment-granules which display rapid movements and later collect at the center of the parasite, at which time the pigment becomes quiescent; while coincidentally peculiar movements are to be seen along the margin of the organism. Almost instantaneously a protrusion or protrusions appear from the edge of the parasite, which elongate, forming thin, colorless, actively motile filaments that are seen lashing among the red cells (Fig. 58, *A*, *B*). The flagellum is apparently continuous with the border of the parasite, and may, in addition to being clubbed at

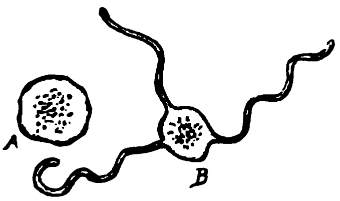

Fig. 58.—*A*, Parasite just prior to flagellation; *B*, flagellated parasite (Craig).

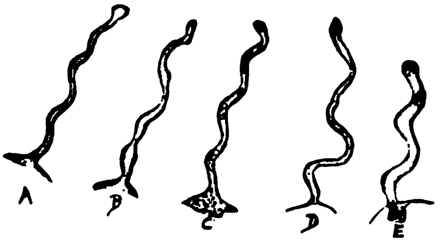

Fig. 59.—Types of flagella: *A*, Clubbed extremity; *B*, nodular swellings; *C*, pigment within flagellum and at extremity; *D*, pigment at extremity; *E*, short, thick, and pigmented (after Craig).

its distal extremity, present one or more nodular expansions and a few pigment-granules (Fig. 59). In length the flagellum usually equals about thrice the diameter of the organism from which it was developed, and from four to eight times that of a red corpuscle. They may be even longer, and short flagella with sluggish serpentine movements may be seen.

All flagella display an active vibratory and often whip-like movement, which displaces the red cells suspended in the plasma, resembling somewhat in this respect, yet in a lesser degree, the effect of the filaria upon the living corpuscles. After a time the flagellum is detached from the body of the parasite, and may be seen coursing through the field in a more or less serpentine manner, traveling rather rapidly between the red cells. This active serpentine movement becomes lessened after a time, and is eventually lost, the flagellum often becoming entangled among the red cells. The accompanying figures (60, 61), compiled from Craig's exhaustive illustrations of malarial flagella developing from the various forms of malarial parasites,* will serve to illustrate the slight differences known to exist.

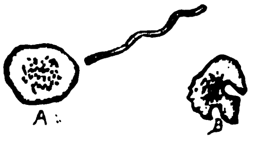

Fig. 60.—*A*, Free flagellum just escaped from parasite; *B*, shrunken state of the parasite after exflagellation (after Craig).

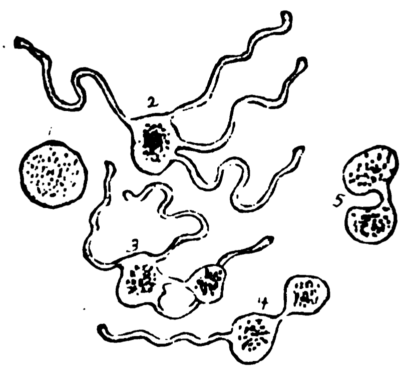

Fig. 61.—1, Prior to flagellation; 2, parasite showing four flagella; 3, fragmentation, dividing into two portions; 4, further fragmentation; 5, fragmentation complete (after Craig).

Termination of Flagella.—(1) The flagellum may become detached from the parent cell; (2) become motionless and disappear; (3) coil around the parasite, which becomes shrunken and degenerated; or (4) the parasite may become fragmented and the flagellum degenerate and disappear.

In the first instance, where the flagellum has succeeded in detaching itself from the parent cell, it swims serpent-like among the corpuscles (Fig. 62, 6). Should a single flagellum be developed from a parasite, the pigment of such parasite becomes quiet after the flagellum is detached, and the cell itself soon becomes shrunken (Fig. 62, 7).

In the study of flagellated malarial parasites one should observe the extreme activity of the pigment in the parasite prior to flagellation and while the flagella remain attached, also the clubbed extremities of the flagella (Fig. 62), as well as their power of individual movement in the blood.

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* "New York Med. Jour.," Dec. 23, 1899, p. 913.

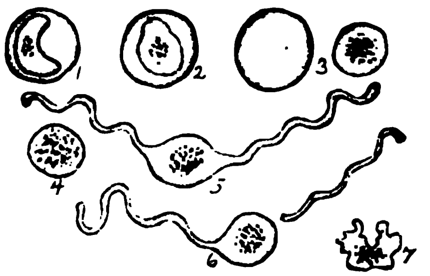


Fig. 62.—Parasite of estivo-autumnal fever: 1, Crescent within the red cell; 2, crescent cell expanded into round body, but within the red blood-cell; 3, round body which has escaped from the red corpuscle, which rests beside it; 4, round body with active pigment; 5, round body putting forth two flagella; 6, round body showing one flagellum detached; 7, shrunken and degenerated parasite (after Craig).

Passive Flagellation.—Not all cases of estivo-autumnal fever exhibit flagellation, since flagella develop from the crescentic bodies; yet it is in this form of fever that we commonly find the so-called "passive flagellated parasite." In addition to crescentic bodies there are also to be seen numerous round and oval bodies whose pigment is distributed in the form of a wreath (Figs. 63, 64). These bodies, according to their pigment-granules, are divided into two forms: first, those in which the pigment is in the form of fine rods and dots, and may at times be motile, again quiescent; second, those forms which are few in number, nearly always round, and whose pigment is in the form of large, black-brown dots seldom displaying movements. The active flagellated organism, previously described (Fig. 61), may arise from the first variety, while from the second variety the passive flagellated organism springs. Flagella developing from this latter variety of parasites are usually few in number, rarely present clubbed extremities, though there is an expanding portion at the junction of the flagellum and the mother-cell (round body), and instead of the active, lashing movements previously described, there is

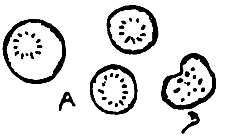


Fig. 63.—*A*, Round body with pigment in form of a ring; *B*, pigment disseminated and feebly motile at times (Craig).

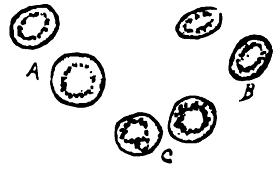


Fig. 64.—*A*, Round bodies with pigment in form of a wreath; *B*, oval bodies showing wreath arrangement of pigment; *C*, round bodies with pigment in the form of dense aggregations (Craig).

to be seen a straightening, relaxing, and apparent revolving of the flagellum upon its axis (Fig. 65). According to Craig, they may detach themselves from the round body and again attach themselves to it. During these maneuvres the pigment-granules of the round parasite remain in the form of a circle, and are usually motionless. This form of flagellation also occurs in tertian fever.

Function of the Flagellum.—The flagellum represents the male element in the cycle of the sexual reproduction of the malarial parasite—flagellation takes place outside the human body and normally within the stomach of the mosquito. To MacCallum, of Baltimore, is due the discovery of the flagellum's function. In a manner somewhat analogous to that of the spermatozoon the detached flagellum penetrates a fully matured malarial parasite which has not yet undergone segmentation. The parasite thus fertilized in the mosquito's stomach penetrates the insect's stomach-wall and here develops into a small cyst which in about seven days displays numerous irregular, ray-like striæ (see *Life-Cycle of Malarial Parasite*, Fig. 66). Later the capsule of this cyst ruptures, and the delicate, thread-like bodies which the cyst contains are liberated in the body of the mosquito, and later many of them are deposited in the insect's salivary glands. A mosquito thus infected, upon biting a healthy human being in addition to sucking blood from the individual, introduces into his system the small thread-like bodies previously stored up in its salivary glands; these bodies being the product of sexual fertilization of the fully developed spermatozoon (flagellum), which fertilization took place in the mosquito's stomach after the insect had satiated its appetite with the blood of a person infected with malaria. The thread-like bodies (Fig. 66, U), introduced into the human system through the mosquito's bite are now properly environed

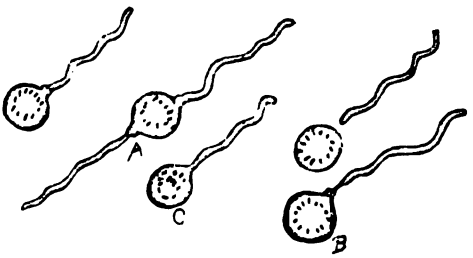


Fig. 65.—Passive flagellation of the estivo-autumnal parasite. Note arrangement of pigment; flagella not clubbed at ends, and straight in outline. One flagellum about to enter round body (after Craig).

for their further development into the hyaline bodies previously described. This final step completes the life-cycles of the malarial

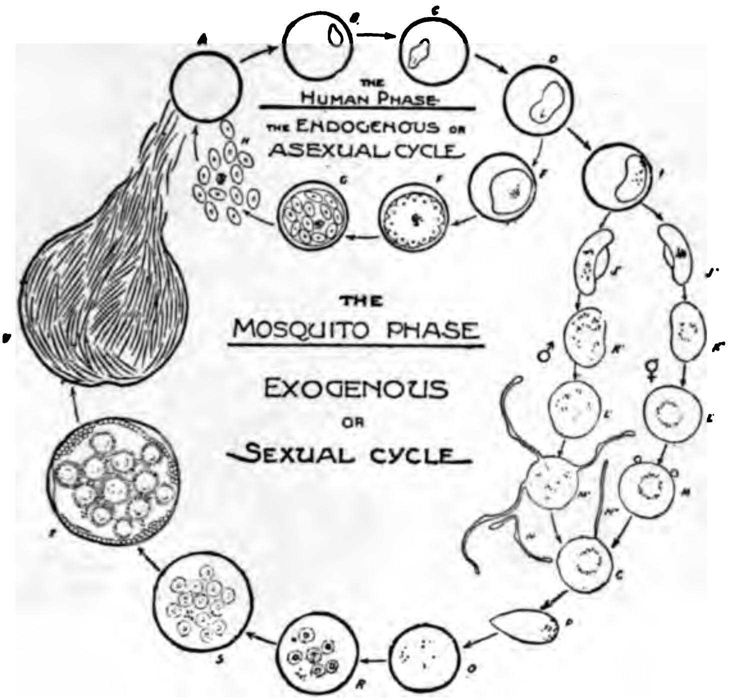


Fig. 66.—Schema showing the human and mosquito cycles of the malarial parasite: A, Normal red cell; B, C, D, E, red cells containing amebulas or myxopods; F, G, H, sporocytes; J′, K′, L′, M′, microgametocytes or male gametes; J″, K″, L″, M″, O, macrogametocytes or female gametes; N′, N″, microgametes; P, traveling vermicule; Q, young zygote; R, S, zygotomeres; T, blastophore; U, mature zygote (modified from Blanchard's diagram illustrating life-cycle of Coccidium schubergi) (Rees, in "Practitioner," March, 1901).

parasite in both its host—man—and the intermediary host—the mosquito. More than one variety of parasite may be found in the blood of man at the same time.

For a description of the Leishman-Donovan body see p. 525.

CHAPTER II.

THE URINE.

It is of utmost importance that the physician have an extensive knowledge of this secretion, since its many variable changes are often expressions of morbid conditions in either the kidneys themselves or elsewhere in the body; an accurate interpretation of these changes provides valuable aid to diagnosis; therefore the study of the urine has now become one of the essential features in clinical diagnosis.

PHYSICAL PROPERTIES OF THE URINE.

NAKED-EYE APPEARANCE.

Quantity.—The quantity of urine secreted by a healthy man in twenty-four hours is from 1500 to 2000 c.c. (42.2 to 52.7 fl. oz.); although 1200 c.c. (33.8 fl. oz.) may at times be the normal quantity where but few liquids are taken. Marked variations are commonly observed, and, as a rule, bear direct relation to the quantity of fluids taken, and the activity of the bowels or the skin; therefore, any excess or deficiency in this quantity secreted should be regarded as pathologic only when extreme. The rate of secretion varies with barometric conditions (temperature and humidity), and is increased during the early part of the night and early morning; while during the hours usually occupied for sleep and during sleep it is lessened.

Under pathologic conditions we often observe wide deviations from the normal standard, and in order to arrive at definite conclusions the urine voided during the twenty-four hours should be collected. The patient should void all the urine possible before going to stool, thus diminishing loss during the act of defecation. When the patient suffers from incontinence, the quantity is approximately estimated by frequent catheterization; yet this procedure is open to many objections, as is also the permanent application of a catheter. Care must be taken to empty the bladder before any of the urine for the twenty-four hours is collected. Collect the urine in a graduated vessel of 2000 c.c. (52.7

fl. oz.) capacity, in order that the quantity may be estimated. When further study is desired, keep the urine in a cool place to prevent decomposition, which is destructive to many of its elements.

Oliguria.—A diminution in the quantity of the urine secreted is a feature accompanying high fevers, interference with the renal circulation,—as in chronic heart disease,—cirrhosis of the liver, hydroperitoneum, and acute and certain forms of chronic nephritis.

Anuria.—Complete suppression of the urine may accompany uremia and conditions where large quantities of liquids are extracted from the body: *e. g.*, Asiatic cholera, dysentery, hemorrhage, and grave anemia. It also follows the administration of large doses of arsenic and oxalic acid (*toxic anuria*). Suppression of the urine may follow profuse perspiration (*physiologic anuria*).

Polyuria.—A notable increase in the quantity of the urine is observed in chronic interstitial nephritis, amyloid disease of the kidneys, during the convalescence from acute nephritis, acute fevers, and in diabetes mellitus and insipidus. It is also present after hysteric seizures and in certain organic brain conditions; in cardiac disease after compensation is re-established; during the absorption of fluids from the serous sacs; and between the paroxysms of relapsing fever. It may also follow the administration of drugs; *e. g.*, digitalis and caffein.

Color.—Normal urine is of a straw or wine-yellow color. Marked variations from the normal are seen, extending from that of a clear watery urine, of low specific gravity, containing little coloring pigment and a comparatively small quantity of solids, through the successive shades to that of a reddish-brown urine, of high specific gravity, rich in both coloring-matter and solids. The pigments usually regarded as accountable for the color of the urine are uroerythrin, pigment of pink urates, normal urobilin, pathologic urobilin,—present during seizures of pyrexia and in grave anemia,—urohematoporphyrin (derivative of hematin), and alkapton, the color of which appears after exposure to air and light. The reaction exercises an influence upon the color of the urine; *e. g.*, acid urines are, as a rule, darker than are alkaline urines, and should an acid reaction persist, the color of the urine deepens upon standing.

Normal urine is of a golden- or amber-yellow color, while highly colored urines may vary through the successive shades to a reddish yellow or red color, and dark urines from brownish red to reddish brown.

Color in Disease.—Pathologic urine may present a pale color and high specific gravity, or vice versâ. Here the color of the urine may depend upon the secretion of additional coloring-matter. Wide variations, depending on the stage of the disease at which the urine is examined, are to be observed; *e. g.*, the urine secreted on the second day of an attack of acute nephritis is of high color, scanty, and of high specific gravity; while the urine at the beginning of convalescence is pale, increased in quantity, and of a low specific gravity.

Highly colored urine is noted in fevers, conditions causing congestion of the kidneys—as in chronic heart disease, pernicious anemia, sarcoma, and cancer of the gastro-intestinal tract. Products of pathologic urobilin, indigo, melanin, or anemia, when contained in the urine in sufficient amounts, may, on the addition of some reagent, develop color. On standing, the urine of phthisis has a tendency to become dark, and, at times, black. Such changes are often due to the development of fungi, and are produced experimentally by the Aspergillus fumigatus and A. niger.

Polyuria.—Polyuria resulting from diabetes insipidus and interstitial or amyloid changes in the kidneys follows the usual rule of diminished pigments and solids; while, on the contrary, the urine of diabetes mellitus is of high specific gravity and as a rule of an amber color.

Chyluria.—Chyluria, parasitic or from other cause, lends to the urine a milky appearance due to the addition of globules of fat.

Bloody Urine.—Urine of a reddish, carmin, or blackish color usually contains an admixture of blood, and varies in color proportionate to the quantity added. Bile-pigment lends to the urine a brownish-yellow color, and if present in large amounts a greenish tint is observed: on shaking, a heavy yellow foam collects on the surface. Urine rich in urobilin is brownish red in color and may on shaking also display a froth. Indoxyl sulphate may cause a dark-brown hue. Indigo is responsible for the blue or dull blue color in the urine.

Green Urine and Drugs.—Green urine is rarely seen and may be due to the presence of biliary coloring-matter (greenish yellow or greenish brown); to large amounts of sugar, cystin, development of bacteria (Bacillus pyocyaneus), and to the administration of methylene-blue. The latter is a common cause of bottle-green urines in the insane department of the Philadelphia Hospital, where the drug is employed as a sedative. Carbolic acid induces a brown color which on standing changes to a blackish brown; a similar effect being produced by naphthalin, resorcin, hydroquinon, and pyrocatechin. Rhubarb and senna cause a brown or red color, while santonin induces a yellow tint; quinin, antipyrin, and sulphonal intensify the color of the urine. The coloring pigments of the urine are doubtless many in number, among which hematoporphyrin is seen.

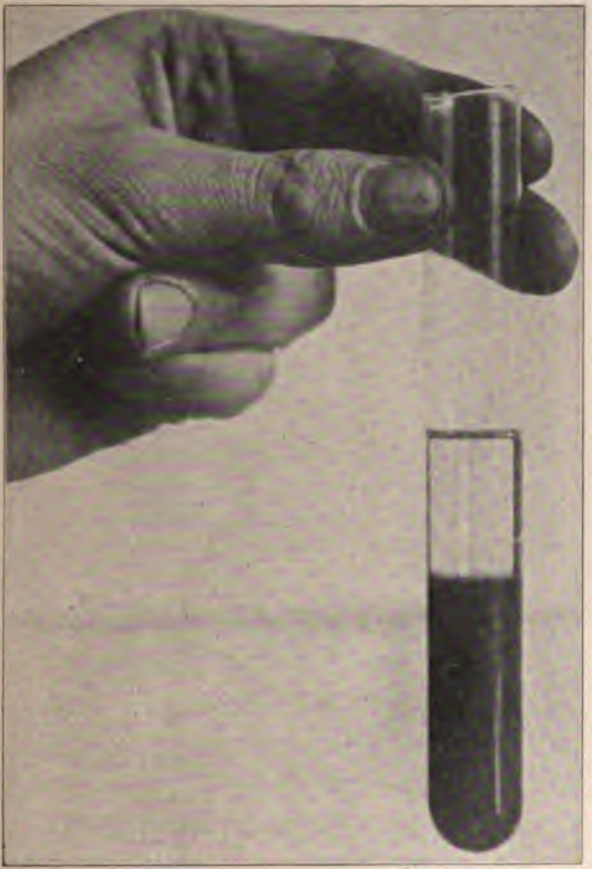


Fig. 67.—Urine turbid, due to urates. Upper stratum heated.

Transparency.—Normal urine is clear when voided, and upon standing it usually becomes cloudy, but is not affected by alkalis, mineral acids, or by heat. Pathologic urine may be cloudy when voided, or may become so, owing to precipitation of urates, phosphates, carbonates, blood, pus, mucus, chyle, or bacteria. Turbidity due to phosphates clears by the addition of acids, and that induced by urates, by the application of heat (Fig. 67). Should the turbidity increase when acid and heat are applied, it is due to albuminous products or to earthy phosphates (see *Scheme*, page 261). Turbidity due to mucus or bacteria is but moderately influenced by acetic acid.

Odor.—The odor of fresh normal urine is that of new hay; decomposition causes the odor of ammonia. Urine containing acetone has an odor of fruit; while that of diabetes gives an aromatic odor. Asparagus, onions, and turpentine in turn lend their characteristic odors to this secretion. Blood when in alkaline urine induces a heavy, putrid odor.

Consistence.—Normal urine is of an aqueous consistence. Urine is thickened by large amounts of sugar, mucus (syrupy), pus, molecular fats, Bence-Jones' albumose, and also, in certain febrile conditions, as the result of an excess of inorganic substances. Alkalinity may also increase the consistence, this consistence being rarely found to be that of gelatin.

SPECIFIC GRAVITY.

The standard for normal urine, when the entire quantity has been collected for the twenty-four hours, can safely be set at 1.020. It usually varies in inverse ratio to the quantity secreted, oscillating between 1.015 and 1.025 when 1200 to 1500 c.c. (33.8 to 42.2 fl. oz.) are voided daily.

Increase.—Nitrogenous foods, fasting, sedentary habits, and pathologic states cause increase; while the taking of large quantities of liquid into the system diminishes the specific gravity.

Urines rich in urates, chlorids, phosphates, oxalates, and sugar display a high specific gravity. An increase is also observed where the quantity of urine passed is lessened; *e. g.*, in acute fevers, acute and chronic nephritis, after mineral poisons, eclampsia, uremia, and following a dry and highly albuminous diet.

Caution.—The specific gravity bears no relation to the nitrogenous constituents of the urine, yet increased nitrogenous elimination and an increased elimination of chlorids are commonly associated, the latter inducing a high specific gravity.

Decrease.—A marked diminution in the specific gravity of the urine may be the first evidence that the kidneys are incapable of performing their function. In chronic nephritis a diminished specific gravity often precedes an attack of uremia; yet the quantity of urine secreted may be normal or even increased. In diabetes insipidus, amyloid kidney, chronic interstitial nephritis, and after the excessive inhibition of malt liquors, the specific gravity is lowered. I found it to be 1.004 in Bence-Jones' albumosuria, a condition where a low specific gravity is common.

In acute fevers where the specific gravity has been high, a sudden diminution signifies either that tissue changes are diminished, their products not formed, or that those processes are active and their products not elaborated by the kidneys; however, it may be the first evidence of a fatal termination. A series of cases studied at the Philadelphia Hospital showed that in acute fevers, chronic nephritis, and in acute alcoholism, a marked lessening in the specific gravity, without change in quantity of the urine, often preceded severe nervous symptoms, which were frequently accompanied by oliguria.

Estimation of the specific gravity is readily obtained by the use of the urinometer (Fig. 68), which should always be furnished with a thermometer, as such instruments are standardized at a given temperature. (For every seven degrees above the temperature at which the urinometer is standardized add one degree to the specific gravity.)

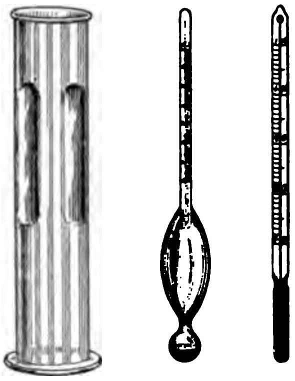


Fig. 68.—Squibb's urinometer.

Method.—Fill a cylindric graduate (Fig. 68) two-thirds full of urine, and remove all froth by touching to it a slip of filter-paper. Introduce the urinometer into the graduate and gently force it to sink to near the bottom of the fluid, when it will be seen to rise and fall until it seeks its level. The figure at the surface of the urine represents the specific gravity of the specimen under examination. Should the quantity of urine be too small for determination of its specific gravity by the urinometer, place the urine at hand in a small cylinder and proceed in the determination by means of "specific gravity beads" (Fig. 69), which are graduated from 1.002 to 1.050. Allow several of the beads to fall gently into the urine and read the specific gravity from the surface of the bead that remains suspended at the center of the column of urine (Fig. 69).

Caution.—The beads should be kept in clear water and cleansed by changing this water after their use. The use of the pyknometer, while insuring accuracy, is not practical for daily clinical work.

Pathologic urine may vary from 1.002 to 1.045 or even higher.

The specific gravity of the urine is of importance, since it bears an approximate relation to the metabolic changes; however, where the kidneys' function is impaired, products resulting from metabolic changes, as uric acid and urea, may be formed in normal amounts and still be retained in the blood and tissues, thereby diminishing the urine's specific gravity.

Solids of the Urine.—The quantity of solids excreted through the urine in a healthy individual of average weight and habits during the twenty-four hours is estimated at 945 grains (61.14 gm.). After the fortieth year the amount of solids will be found to diminish gradually; and to meet this reduction deduct 10 per cent. from the above standard during the decad between forty and fifty; and an additional 10 per cent. for each successive decad. Conditions altering the amount of solids excreted are largely those influencing the specific gravity of the urine.

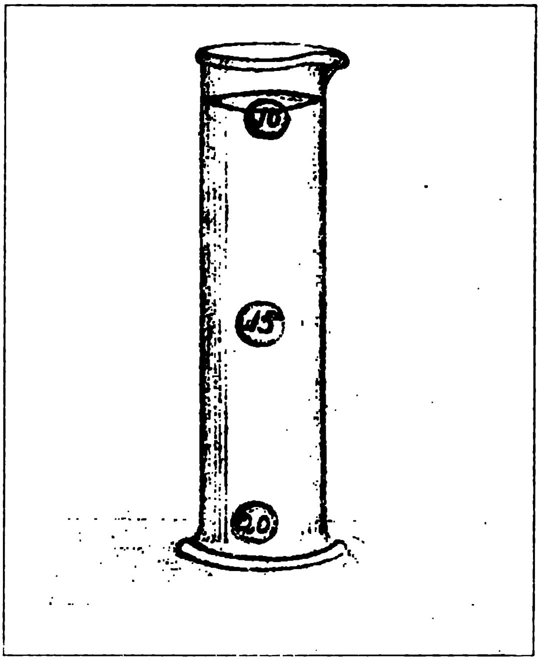


Fig. 69.—Use of beads for determining the specific gravity of small quantities of urine and other fluids.

Computation.—The total amount of solids is estimated by multiplying the last two figures of the specific gravity by 2.33, the co-efficient of Haeser; *e. g.*, the urine voided during the twenty-four hours is found to be 1800 c.c., and the mixed product has a specific gravity of 1.020. Therefore $20 \times 2.33 = 46.60$, the amount of solids estimated in grams present in 1000 c.c. of such urine, and $\frac{46.60 \times 1800}{1000} = 83.8$, the number of grams of solids suspended in the urine voided during the twenty-four hours. The clinical value of estimating the total amount of solids has certain limitations, for which reason more elaborate methods are omitted (see *Chemistry*, page 177). It should be emphasized that the determination of the total solids of a given specimen voided at one urination is valueless, since the specific gravity fluctuates between wide limits at different hours of the day.

ELECTRIC CONDUCTIVITY OF THE URINE.

Electric conductivity of the urine in relation to its chemic composition is among the newer applications of modern physiologic chemistry, and is of importance when employed in the solution of problems in both physiology and physiologic chemistry. It is fair to believe that it will add greatly to our present laboratory methods for the ascertainment of pathologic states of the urine. (Electric conductivity may also be applied to other body fluids—pleural and peritoneal. It has been shown by Roth,[*] Stewart,[†] Bugarszky,[‡] Tangl,[§] and a number of other observers that this method is applicable to the examination of both blood-serum and serous fluids. They have further found that the inorganic constituents of blood-serum are much more constant than are the inorganic constituents of urine.) The extended variations in the conductivity of urine cause one at first sight to regard them as being of little clinical moment. Bugarszky[‖] has shown that certain general relations exist between the total ash of the urine and the conductivity, showing that the conductivity of the urine is practically due to the mineral salts held in suspension.

Urea.—Most abundant of the urinary constituents is the urea, which is nearly a non-conductor when thus suspended in solution, and it does not, therefore, materially influence our results. Kreatinin, ammonia, xanthin bases, uric acid, and urates have but slight conductivity. The conductivity of the urine and the specific gravity when studied correlatively will be found to have a value somewhat analogous, the former being slightly the more

[*] "Centralbl. f. Physiologie," Nov. 27, 1897.

[†] *Ibid.*, p. 332.

[‡] *Ibid.*, p. 297.

[§] *Ibid.*, p. 301.

[‖] "Pflüger's Archiv f. die gesammte Physiologie," 1897, vol. lxviii, p. 389.

definite, which renders electric conductivity of value in estimating the degree of metabolism of inorganic substances—a point which has been ably demonstrated by J. H. Long* who has also elaborately described the special technic necessary for the application of this method, as have also other writers to whom I have referred.

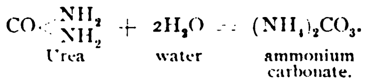
REACTION OF THE URINE.

Healthy mixed urine from the twenty-four hours' product is acid, due to the presence of diacid sodium phosphate (NaH_2PO_4). Such acids as uric, sulphuric, carbonic, and hippuric are thought to take up a portion of the sodium from the urine, leaving behind an acid salt. Taylor estimated the acidity of the normal urine for twenty-four hours as equivalent to 14 grains of carbonate of sodium or 30 grains of oxalic acid.

Decreased Acidity.—Conditions lessening the degree of acidity are a full meal, a diet largely vegetable, ingestion of foods rich in vegetable acids or their salts, such as acetates, lactates, and tartrates, alkaline carbonates, free perspiration, and paroxysms of vomiting. Temporarily diminished acidity is observed in pneumonia during the forenoon (Quincke) and twenty-four hours after the crisis. Standing lessens the acidity through decomposition of the urate of sodium.

Increased Acidity.—The acidity is increased by allowing the urine to stand for a few hours, and is due to an increase of lactic and acetic acids, acid phosphates, and probably to the formation of new acids from such pre-existing substances as alcohol and carbohydrates (diabetic urines). This acid stage is of short duration and the urine is rendered alkaline by the action of bacteria, as well as by chemic changes. Muscular exercise, a diet largely of meats, and the taking of mineral acids increase the urine's acidity, and high acidity is a feature of acute fevers, scurvy, phthisis, diabetes, and leukemia.

Alkaline Reaction.—Pathologic urine may be alkaline when voided, due to ammoniacal fermentation of uric acid and the breaking-up of urea into ammonium carbonate. Thus:



$$\underset{\text{Urea}}{CO\genfrac{}{}{0pt}{}{NH_2}{NH_2}} + \underset{\text{water}}{2H_2O} = \underset{\text{ammonium carbonate.}}{(NH_4)_2CO_3.}$$

Such a condition implies that there are pathologic changes along the urinary tract, commonly the bladder.

Method of Detecting Reaction.—The reaction of urine is taken by means of red and blue litmus-papers, blue litmus being turned red by acid urines, while alkaline urines change red litmus to blue. The litmus should be cut into narrow strips and kept in a well-stoppered glass jar. Immerse the slip in water immediately before use and then dip one-half inch of its tip into the urine, when the change induced by the urine will appear.

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* "Jour. of the Amer. Chem. Soc.," Oct., 1902, vol. xxiv, No. 10.

Amphoteric Reaction.—The same urine may at times change red litmus to blue and blue litmus to red (amphoteric). This peculiar reaction depends upon the presence of both acids and neutral sodium phosphates, which substances are eliminated simultaneously and equally.

Fixed and Volatile Alkalis.—To distinguish between alkalinity due to fixed alkali (sodium or potassium) and that due to a volatile alkali (ammonia), red litmus-paper, which has been turned blue by the alkalinity of the urine, is dried by gentle heat. No change is observed when a fixed alkali is present, but if the alkalinity depends upon a volatile alkali (ammonia), the litmus surrenders its blue color and displays a reddish or yellow tint.

CHEMISTRY OF THE URINE.

The exact chemic composition of the urine is by no means constant, but fluctuates within rather wide limitations even in the apparently normal individual; and these variations depend, in great measure, upon such factors as exercise, age, sex, cold or hot baths, diet, temperature, digestion, and sleep. A fair classification of the constituents of healthy urine has been suggested by Parkes and is shown by the following table:

Constituents.	Weight. 66 Kilograms.	Per Kilogram of Body-weight.
Water	1500.00 gm.	22.730 gm.
Total solids	72.00 "	1.090 "
Urea	33.18 "	0.500 "
Uric acid	0.55 "	0.008 "
Hippuric acid	0.40 "	0.006 "
Kreatinin	0.91 "	0.014 "
Pigment and other organic matters	10.00 "	0.151 "
Sulphuric acid	2.01 "	0.030 "
Phosphoric acid	3.16 "	0.048 "
Chlorin	7–8.00 "	0.126 "
Ammonia	0.77 "	
Potassium	2.50 "	
Sodium	11.09 "	
Calcium	0.26 "	
Magnesium	0.21 "	

Becquerel's estimation for each 1000 grains of urine is, water 967 grains; solids 33 grains. Of the solids, 24.865 grains were organic matter, leaving but 8.135 grains of inorganic substance.

During certain pathologic conditions the urine may hold in solution, in variable amounts, many substances, among which are: serum-albumin, serum-globulin, peptone, albumose (Bence-Jones'), nucleo-albumin (mucin), glucose, dextrin, lactose, inosite, blood-pigments, bile-pigments and bile acids, melanin, leucin, tyrosin, oxybutyric acid, lecithin, allantoin, fat, acetone, cholesterin, urocaninic acid, sulphureted hydrogen, cystin, and alcohol.

MINERAL ASH.

Quantitative Estimation.—1. Fifty c.c. of urine are placed in a porcelain dish, the weight of which has been taken. It is then evaporated to dryness at a temperature of 100° C. Heat until no gas is evolved.

2. The residue is now taken up by distilled boiling water and later filtered through a filter the weight of whose ash is known.

3. Both dish and contents of the filter are washed with hot water, and the dish and filter dried in an oven; after which the filter is placed in the dish and slowly incinerated.

4. When the dish becomes white, the washings and filtrate which have been set aside are now added to the ash and evaporated at 100° C. Prolong the heat over the flame—care being taken lest too great heat be applied and certain substances be volatilized (chlorin), and sulphates converted into sulphids in the presence of organic matter.

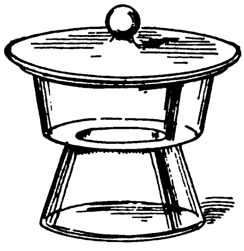

Fig. 70.—Desiccator.

5. Cool the dish in a desiccator (Fig. 70) and ascertain its weight. The difference between its present and previous weights equals the quantity of ash in 50 c.c. of urine.

Cautions.—Place 4 to 6 ounces of dried desiccated (lump) calcium chlorid in the bottom of the desiccator. This keeps the interior of the jar dry and absorbs any moisture present. The lid of the desiccator is ground to fit the jar, and should be hermetically sealed by smearing the edge (ground surface) of the lid with equal parts of paraffin and vaselin. A desiccator provided with a special shelf for the calcium may be obtained.

CHLORIDS.

Chief of the alkalis found in the urine is sodium in combination with chlorin, 10 to 15 gm. being excreted by a healthy man during twenty-four hours. Chlorin in combination with calcium, magnesium, potassium, and ammonium is found in small proportions. In health, the amount of sodium chlorid excreted is directly influenced by the quantity and quality of food ingested.

Decrease.—The chlorids are decreased in starvation, and temporarily after taking freely of liquids, in certain febrile conditions, notably typhoid fever, scarlet fever, roseola, variola, typhus fever, acute yellow atrophy, and they may be absent in croupous pneumonia. A correspondingly marked decrease is seen in acute and chronic nephritis when associated with albuminuria; also in chronic lead intoxication, pemphigus, impetigo, and the early stage of dementia. Again, it is visible in chronic gastro-intestinal conditions, as gastric ulcer, cancer, dilatation of the stomach, and in diarrhea. A less marked decrease is noticed in the anemias, rickets, chorea, idiocy, and melancholia.

Increase.—The chlorids are increased after the administration of potassium salts, during the absorption of exudates and transudates, following epileptic seizures (polyuria), diabetes insipidus, when they have been retained in acute febrile conditions, and during and immediately after the paroxysm in intermittent fever.

Detection of Chlorids.—*Reagents Required.*—1. Nitric acid. 2. Solution of silver nitrate (1 dram of the crystalline salt to the ounce).

The above solution of nitrate of silver, when added to albumin-free urine, is capable of precipitating the chlorids where the urine is first acidulated by adding a few drops of nitric acid. This precipitate appears as an opaque, milky-white chlorid of silver. Normal urine contains from ½ to 1 per cent. of chlorids.

Application.—1. Ten to 15 c.c. of urine free from albumin (see *Albumin*, page 206) are placed in a test-tube, and to it a few drops of nitric acid are added, the tube being then shaken.

2. Add, drop by drop, the silver solution, noting carefully any changes that occur.

Each drop of the silver solution causes a curdy, white clump to fall to the bottom of the tube, this mass in normal urine not becoming disseminated upon shaking the tube, nor does it tend to make the entire liquid milky.

Should the chlorids be reduced to 0.1 per cent., the drop of silver solution merely causes an opalescence; while in the absence of chlorids no change is observed. A copious precipitate is indicative of an increase in the quantity of chlorids present. The precipitate due to chlorids is soluble in ammonia, but insoluble in nitric acid.

Quantitative Estimation of the Chlorids.—Salkowski's Modification of Volhard's Method.—*Reagents Required.*—1. Pure nitric acid, specific gravity 1.2.

2. Concentrated solution of double sulphate of iron and ammonia free from chlorin (may be freed from chlorin by recrystallization).

3. Saturated solution of nitrate of silver (29.075 gm. to the liter); 1 c.c. of such solution corresponds to 0.01 gm. of sodium chlorid. The crystallized salt should be employed.

4. Sulphocyanid of potassium or of ammonium dissolved in water $6\frac{5}{7}$ gm., adding water sufficient to make 400 c.c. (2.5 c.c. of this solution correspond to 10 c.c. of the standard silver solution).

The following method may be employed for standardizing the sulphocyanid solution:

Ten c.c. of silver solution (3) are placed in a flask and 90 c.c. of water added. Four c.c. of nitric acid (1) are now added; and finally 5 c.c. of the double sulphate solution (2). After the mixture is well shaken, the sulphocyanid of ammonium solution is carefully added from the buret until a slight red color appears. This process should be repeated often, noting at each time the quantity of sulphocyanid solution necessary, and the mean obtained. Based upon these results the sulphocyanid solution is diluted to a point so that 2.5 c.c. correspond to 1.0 c.c. of the silver solution. Should the reaction (red color) appear after 20 c.c. of the sulphocyanid solution are added, the following formula serves to determine the quantity of water to be added to one liter —$20 : 25 :: 1000 : x$ (x equals 1250), therefore 250 c.c. of water are added in order that 25 c.c. shall correspond to 10 c.c. of the silver solution.

Application.—1. Ten c.c. of the urine previously freed from albumin are placed in a flask of 100 c.c. capacity.

2. Add 50 c.c. of water.

3. Then add 4 c.c. of the nitric acid (1) and follow with an excess (15 c.c.) of the silver nitrate (3).

4. The mixture is now vigorously shaken until the precipitation is completed and the fluid begins to clear, when enough water is added to make 100 c.c.

5. Filter through a dry paper into a flask holding 80 c.c.

6. These 80 c.c. of fluid are now transferred to a flask of 250 c.c. capacity.

7. Five c.c. of the solution of the double sulphate of iron and ammonia (2) are added.

8. The sulphocyanid solution of ammonia (4), 25 c.c. of which equal 10 c.c. of silver nitrate (3), is slowly added from the buret (Fig. 71) until the terminal reaction (a red color) is obtained and does not disappear on shaking.

The excess of silver solution not needed to precipitate the chlorids from 10 c.c. of urine is now estimated volumetrically by the sulphocyanid of ammonium solution, 2.5 c.c. of which correspond to 1.0 c.c. of the silver solution: Therefore should 12.5 c.c. of sulphocyanid solution be required to produce the terminal reaction, then there were 5 c.c. of the silver solution not employed in precipitating the chlorids from the 10 c.c. of urine. Since 15 c.c. of silver solution were added and 5 c.c. were found to be in excess, it required 10 c.c. to precipitate the chlorids from 10 c.c. of urine.

Again: 1 c.c. of silver solution corresponds to 0.01 gm. of sodium chlorid, and since 10 c.c. of silver solution have been required, 0.1 gm. of sodium chlorid being present in the 10 c.c. of urine, 100 c.c. would therefore contain 1.0 gm. Judging, for example, the twenty-four hours' product to have equaled 800 c.c., there would therefore have been excreted during the twenty-four hours 8 gm. of sodium chlorid.

Fig. 71.—One-hundred c.c. buret.

PHOSPHATES.

Under normal conditions the urine of the twenty-four hours' product will be found to contain from 2.5 to 3.5 gm. of phosphoric acid. This is derived in most part from the food; a small portion is referable, however, to that stored up in proteid molecules in the nerve-cells, muscle-cells, red blood-cells, and the bones. Nerve tissue which is rich in lecithin and in nucleins yields much phosphorus, which is found both in combination with the alkaline earths (earthy phosphates) and with the alkalis (alkaline phosphates). Of these, the alkaline phosphates are in excess by one-third. Sodium enters into combination with the greater portion of the phosphoric acid, potassium, calcium, and magnesium salts in a small amount, and glycerin as glycerin-phosphoric acid only in a trace.

Earthy Phosphates.—Earthy phosphates are phosphates of calcium and of magnesium and exist in the proportion of 33 to 67. There are from 1.0 to 1.5 gm. excreted during the twenty-four hours. They are insoluble in water, but soluble in acids, and are held in solution in acid urines; while in urines alkaline the result of fermentative changes, ammonia is set free by the decomposition of urea, and unites with the acid magnesium phosphates, forming characteristic crystals (triple phosphates). (Fig. 106.)

Caution.—Do not mistake the precipitate which forms upon the application of heat to such urines for that of serum-albumin, which it closely resembles, nor for clouding the result of inflammatory exudates from the urethra or bladder. The opacity caused by phosphates clears upon the addition of acids.

Alkaline Phosphates.—Alkaline phosphates consist of the acid phosphates of sodium and phosphates of potassium—the former being abundant, the latter scant. They are freely soluble in water and in alkalis. Two to 4 gm. are excreted during the twenty-four hours. The acidity of the urine depends upon the quantity of diacid sodium phosphate (NaH_2PO_4) present. This salt also helps to hold in solution the calcium phosphates of acid urines.

As has been previously stated of chlorids, the phosphates are increased by the ingestion of large quantities of liquids, food, animal diet, during starvation and increased tissue destruction, reaching the maximum toward evening, falling gradually, and reaching the minimum about midnight. Morbid conditions may cause either an increase or a diminution in the total amount of phosphates.

Increase.—An increase is noted in leukemia, convalescence from acute fevers, diabetes insipidus, diabetes mellitus (phosphates appearing in inverse ratio to the quantity of sugar), and during the febrile stage of cerebrospinal meningitis, mania (stage of excitement), tabes dorsalis, cerebral tumor, and arthritis deformans.

Drugs.—Such drugs as are cerebral depressants, maximum doses of alcohol, morphin, chloral, chloroform, mineral and vegetable acids, and potassium bromid, also cause an increased elimination, as do hot baths and exposure to a low temperature.

Diabetes.—Phosphatic diabetes has at times been used to distinguish an increased elimination of phosphates reaching a maximum of 9 gm. during the twenty-four hours, and associated with many of the symptoms of diabetes mellitus. Sugar is rarely present.

Decrease.—Phosphates are diminished in acute fevers,—

probably due to retention,—being most marked prior to death; likewise in chronic nephritis, amyloid kidney, anemias, Addison's disease, osteomalacia, major hysteria, chronic rheumatism, acute rheumatism, chronic lead intoxication, acute yellow atrophy, hepatic cirrhosis, and in many of the chronic nervous diseases (hydrocephalus, progressive paralysis, cerebral syphilis). In mania (stage of depression) and in melancholia the alkaline phosphates diminish, while the earthy phosphates increase.

Drugs and Baths.—Certain drugs, as quinin and cocain, are followed by a decrease; while a relative decrease follows the use of strychnin, alcohol, valerian, phosphoric acid, cold baths, and sea-bathing.

Detection of Earthy Phosphates.—Render 10 c.c. of urine alkaline by adding either caustic soda, potash, or ammonia; heat gently, when the phosphates will be precipitated as a whitish cloud which collects at the bottom of the tube. This precipitate is soluble in acetic acid.

Estimation of Earthy Phosphates (Approximate).—Ultzmann's Method.—A special graduated test-tube two centimeters wide is required.

1. This tube is filled with urine to a depth of 5.33 cm.
2. Add a few drops of ammonium or potassium hydrate, and apply heat gently, when the earthy phosphates are precipitated.
3. The tube is allowed to stand for fifteen to twenty minutes, and the depth of the sediment as registered by the tube is taken. A depth of from 1 to 1½ centimeters equals the normal amount. Should the sediment register above or below these points, there is either an increase or a decrease in the earthy phosphates.

Most urines which are alkaline as the result of decomposition display a heavy sediment of earthy phosphates.

Caution.—Do not mistake such sediment for an excess of phosphates, as it is frequently seen in urine deficient in earthy phosphates.

Clinical Significance.—Earthy phosphates are increased in osteomalacia, rickets, rheumatoid arthritis, cerebral disorders, mental strain, and by food and drink. A decrease is observed in renal disease.

Detection and Estimation of Alkaline Phosphates (Approximate).—*Reagent.*—Magnesium fluid (magnesium sulphate and ammonium chlorid, each, 1 part; distilled water, 8 parts; pure liquor ammonia, 1 part).

Earthy phosphates are precipitated by ammonia and readily removed by filtration; but since their clinical importance is limited, their removal is not always required.

Application.—1. Fifty c.c. of the urine are placed in a test-glass and to it 5 c.c. of the magnesium solution are added, shaking gently.

2. A snowy deposit of crystalline ammoniomagnesium phosphate and of amorphous calcium phosphate appears. Should the entire liquid have the appearance of milk, the alkaline phosphates are normal in amount; the appearance of cream indicating an excess, while a faint cloud or opalescence is indicative of a decrease.

Clinical Significance.—The reader is referred to general remarks, page 181.

Estimation of Phosphoric Acid.—*Reagents.*—1. Acetate of soda solution (100 gm. of acetate of soda are dissolved in 800 c.c. of distilled water; 100 c.c. of a 30 per cent. solution of acetic acid are added, and enough distilled water to make 1000 c.c.).

2. Potassium ferrocyanid (saturated solution), or cochineal tincture (a few grams of cochineal in a 25 per cent. solution of alcohol).

3. Uranium solution (take 20.3 gm. of commercial uranium nitrate, purified and well dried, and dissolve in a small quantity of acetic or nitric acid; add distilled water to make 1 liter). One c.c. indicates 5 mgm. of P_2O_5.

Fig. 72.—Nest of beakers.

Application.—1. Fifty c.c. of urine are placed in a beaker (Fig. 72) and 5 c.c. of soda solution (1) added.

2. A few drops of the cochineal tincture (2) are now added, and the mixture heated vigorously over a water-bath.

3. The uranium solution (3) is now gradually added from a buret until a slight, but permanent, green color is established.

The terminal or color reaction develops after all the phosphoric acid has been precipitated by the uranium solution. When ferrocyanid of potassium is used as the indicator, a reddish-brown color appears. Whenever the precipitate ceases to form, the addition of the uranium solution is suspended, the mixture stirred, and a drop placed on a porcelain dish, and to it a drop of ferrocyanid solution added. Should no reddish-brown color appear at the point of contact, the uranium solution and heat are continued.

The number of cubic centimeters of uranium solution employed when multiplied by 0.005 equals the amount of phosphoric acid contained in the 50 c.c. of urine. From this result the quantity eliminated for the twenty-four hours is readily calculated.

SULPHATES.

Sulphuric acid exists in the urine both as mineral (preformed) sulphates and as ethereal (conjugate) sulphates. In a healthy individual the total excretion of sulphates through the urine for the twenty-four hours varies from 2 to 3 gm.—less than one-tenth of which is ethereal sulphates. The quantity of sulphates excreted is greatly influenced both by the amount of proteid substance ingested and by the rapid destruction of the tissues.

Increase.—An increased elimination is observed after a diet of animal proteids, in acute fevers, pneumonia, myelitis, cerebritis, rheumatism, affections of the muscles, and especially in meningitis. In diabetes mellitus, diabetes insipidus, eczema, pseudohypertrophic paralysis, progressive muscular atrophy, and leukemia, an increase appears and is especially marked in the latter condition.

Drugs.—Morphin, potassium bromid, sodium salicylate, and antifebrin also cause an increased elimination.

Ethereal Sulphates.—Ethereal or conjugate sulphates are found increased during certain pathologic conditions associated with diminished hydrochloric acid; hence intestinal fermentation; obstructive jaundice and intestinal obstruction cause an increase. The normal ratio of mineral to ethereal sulphates is as 10 is to 1, and is found to vary widely in disease.

Diminution.—The total sulphates are found diminished when upon a vegetable diet, during convalescence, in chronic renal disease, non-obstructive jaundice, diarrhea, and during starvation. A strongly acid gastric fluid (due to lactic and butyric acids) and alcoholic excesses may cause a diminished elimination of the ethereal sulphates.

Detection and Approximate Estimation.—*Reagents.*—1. Solution of barium chlorid (1 part to 8).

2. Acetic acid (specific gravity, 1.04).

Application.—1. Place 10 c.c. of urine in a test-tube.

2. Acidify with acetic acid.

3. Add about 3 c.c. (one-third volume) of barium chlorid solution—1 c.c. at a time, shaking gently after each addition.

Reaction.—A white, milky precipitate indicates the presence of sulphates in normal amounts; but should the liquid assume the consistence of cream, sulphates are present in excess. If the liquid becomes opalescent, sulphates are diminished.

Quantitative Estimation of Total Sulphates.—*Reagents.*—

1. Hydrochloric acid (specific gravity, 1.12).

2. Saturated solution of barium chlorid.

Application.—1. One hundred c.c. of filtered urine are placed in a beaker and treated with 8 c.c. of hydrochloric acid (1), and heated over a water-bath to boiling.

2. Twenty c.c. of barium chlorid solution (2) are now added, and heat continued until the barium has thoroughly settled as barium sulphate, and the supernatant liquid is clear (occupying from twenty to forty minutes).

3. Filter through a Gooch funnel (Fig. 73) that is provided with a plug of asbestos; all having been dried and weighed.

4. Small quantities of water are to be added to the last cubic centimeter, and the final portion is placed upon the filter by means of a rubber-tipped glass rod.

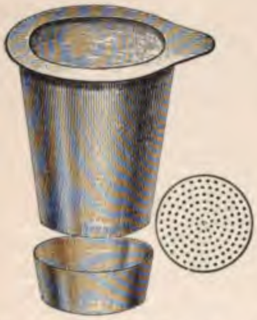

Fig. 73.—Gooch filter or funnel.

Caution.—Never allow the filter to become dry during filtration.

5. Wash the precipitate with boiling water until the wash-water is clear and remains clear after a drop of dilute sulphuric acid has been added.

6. Remove all gum-like substances by washing with hot alcohol (70 per cent.).

7. Fill the filter two or more times with ether. When a paper filter has been employed, it is placed in a porcelain crucible of known weight, and ignited. The crucible is nearly covered by the lid and the ash heated moderately for a time. It is then half covered and heated further until the ash becomes white, and after cooling the crucible is placed in a desiccator and weighed. The difference between the first and second weights equals the weight of barium sulphate found in 100 c.c. of urine.

A portion of sulphate is reduced through combustion in the presence of organic matter, so that the weight obtained is too low; therefore wash the barium sulphate into a beaker with a small quantity of water colored red by a few drops of an alcoholic solution of phenolphthalein. This liquid is titrated with a one-tenth normal solution of sulphuric acid until the red color disappears. Each cubic centimeter of the one-tenth normal solution corresponds to 0.004 gm. of barium sulphate. The figure obtained in this manner is added to that acquired by weighing, and the amount equals the total barium sulphate derived from 100 c.c. of urine.

Quantitative Estimation of the Ethereal (Conjugate) Sulphates.—*Reagent.*—Alkaline solution of barium chlorid.

Dilute hydrochloric acid.

Application.—1. Place 100 c.c. of filtered urine in a beaker; add 100 c.c. of barium-chlorid solution, and stir thoroughly.

2. After standing a few minutes filter into a graduate up to the 100-c.c. mark.

3. Acidify strongly with dilute hydrochloric acid and boil on a water-bath until the barium sulphates which form settle.

4. Filter, wash, dry, and weigh precipitate (see above).

The weight thus gained is multiplied by two and the product equals the ethereal sulphates in 100 c.c. of urine, and when this is deducted from the amount of total sulphate formed, the difference will equal the amount of mineral (preformed) sulphates.

Estimation of Neutral Sulphur.—The oxidized sulphur, mineral and ethereal sulphates are estimated as before described, and the difference between the sum of these and total amount of sulphur equals the amount of neutral sulphur present.

Reagents.—1. Mixture of sodium and potassium carbonate (11 to 14).

2. Potassium permanganate (crystalline).

3. Concentrated hydrochloric acid.

4. Saturated solution of barium chlorid.

Application.—1. One hundred c.c. of urine are treated with 12 gm. of sodium-potassium-carbonate mixture, and evaporated to dryness in a nickel crucible.

2. Fuse the residue, cool, and extract with hot water.

3. Filter off the carbonaceous residue.

4. Treat the filtrate and washings with a few crystals of potassium permanganate and heat for fifteen minutes, when more potassium permanganate is added, and heat continued; should the solution become decolorized, the heat is prolonged fifteen minutes longer.

5. Add concentrated hydrochloric acid until a decided acid reaction is obtained.

6. Boil this solution and treat with 20 c.c. of the barium-chlorid solution, and proceed further as described under the Estimation of Total Sulphates (page 186).

Loosely Combined Sulphur in the Urine.—This is a factor many chemists regard as a pre-eminent feature which distinguishes Bence-Jones' albumose from the digestive albumoses, as well as as from histon and globin (see *Albumosuria*, page 217).

The Author's Method.*—1. Fifteen to 20 c.c.of filtered urine are placed in a test-tube, and to it an equal quantity of a saturated solution of sodium chlorid is added, shaking the tube to insure a mingling of the liquids.

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* "Amer. Jour. Med. Sci.," Oct., 1902, p. 567.

2. Two or three c.c. of a 30 per cent. solution of caustic soda are now added, the shaking continuing vigorously.

3. The upper one-fourth of this column of liquid is gradually heated over a flame to the boiling-point, when a solution of lead acetate (10 per cent.) is added drop by drop, the upper stratum of liquid being boiled after each additional drop.

4. When the drop of lead solution comes in contact with the liquid, a copious, pearly or creamy, cloud appears at the surface, becoming less dense as the boiling-point is neared; and when ebullition is prolonged for from one-half to one minute, the upper portion of the liquid shows slight browning, which deepens to a dull black color, as shown by the accompanying illustration (Fig. 74).

Below this point for some distance is seen a variable degree of browning (2). Standing intensifies the reaction, and if this be prolonged for several hours, the black precipitate falls through the clear stratum of liquid (3), collecting in the bottom of the tube as a coarsely granular pigment. Personally, this method has been employed in the study of four cases of Bence-Jones' albumosuria—dilution, 1 part in 10, and often a much higher dilution gave positive results.

Another method worthy of mention is to place a quantity of urine in a beaker and heat on a water-bath to from 54° to 60° C., then cool and filter. The precipitate collected is dissolved by washing with a solution of soda (specific gravity, 1.16). The precipitate is now heated to the boiling-point, when a 10 per cent. solution of lead acetate is added drop by drop, and the boiling continued. The creamy precipitate which first appears soon shows evidence of browning and changes later to dull black.

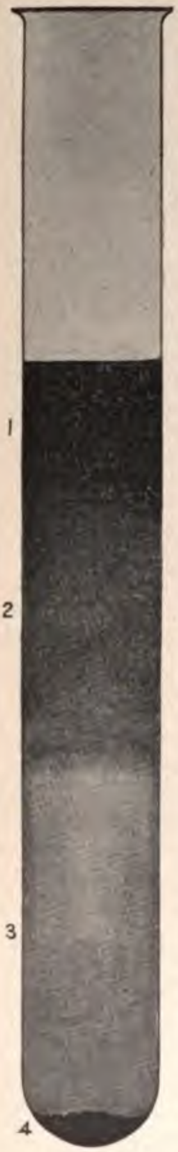

Fig. 74.—Author's reaction for Bence-Jones' albumose.

CENTRIFUGAL ANALYSIS.

This method of determining the quantity of inorganic substances present in the urine, while only approximately estimating the chlorids, phosphates, and sulphates, gives results which approach accuracy as nearly as is required in most clinical work. Its daily use in the author's laboratories has proved it to be of efficient service and worthy of special mention, since it is possible through it to estimate in twenty minutes the inorganic constituents of a given urine.

The equipment necessary is a centrifuge (Fig. 75) with a standard radius of arm and tube, capable of a speed of 1200 revolutions per minute, and Purdy's graduated percentage tubes (Fig. 76).

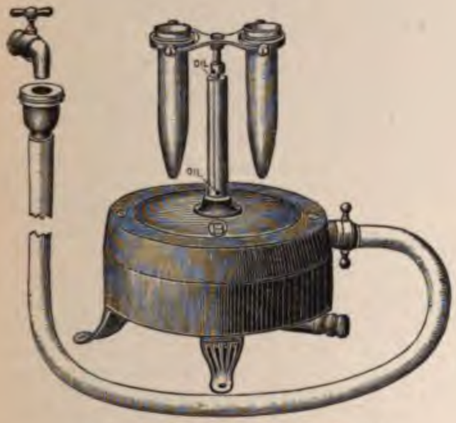

Fig. 75.—Water-motor centrifuge.

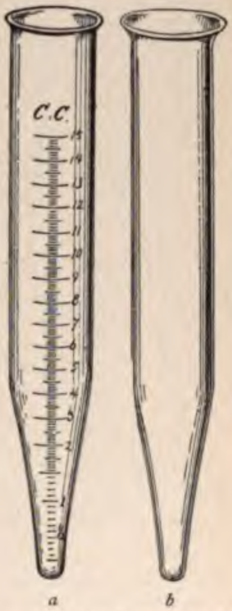

Fig. 76.—Purdy's tubes for the centrifuge: *a*, Percentage tube; *b*, sediment tube.

Estimation of Chlorids.—Fill the tube with filtered urine to the 10-c.c. mark, and add 1 c.c. of strong nitric acid and 4 c.c. of the standard silver-nitrate solution (AgN_3, ʒj, distilled water, ℥j). Place the thumb over the mouth of the tube and invert it three or four times to effect a complete mingling of the urine and reagents. Allow it to stand for three minutes and then place in the centrifuge at the speed indicated for three minutes, when the amount of precipitate is read. By consulting the accompanying table (which was devised by the late Dr. Purdy) the amount of AgCl present can be determined.

TABLE FOR THE ESTIMATION OF CHLORIDS AFTER CENTRIFUGATION.

Showing the bulk-percentage of silver chlorid (AgCl) and the corresponding gravimetric percentages and grains per fluidounce of sodium chlorid (NaCl) and chlorin (Cl).—(Purdy.)

Bulk-percentage of AgCl.	Percentage NaCl.	Gr. Per Oz. NaCl.	Percentage Cl.	Gr. Per Oz. Cl.	Bulk-percentage of AgCl.	Percentage NaCl.	Gr. Per Oz. NaCl.	Percentage Cl.	Gr. Per Oz. Cl.
¼	0.03	0.15	0.02	0.1	8	1.04	4.98	0.63	3.02
½	0.07	0.31	0.04	0.19	8½	1.1	5.29	0.67	3.22
¾	0.1	0.47	0.06	0.28	9	1.17	5.6	0.71	3.4
1	0.13	0.62	0.08	0.38	9½	1.23	5.91	0.75	3.6
1¼	0.16	0.78	0.1	0.48	10	1.3	6.22	0.79	3.79
1½	0.19	0.93	0.12	0.57	10½	1.36	6.53	0.83	3.97
1¾	0.23	1.09	0.14	0.66	11	1.43	6.84	0.87	4.16
2	0.26	1.24	0.16	0.76	11½	1.49	7.2	0.91	4.35
2¼	0.29	1.41	0.18	0.85	12	1.56	7.46	0.95	4.54
2½	0.32	1.56	0.2	0.96	12½	1.62	7.78	0.99	4.73
2¾	0.36	1.71	0.22	1.04	13	1.69	8.09	1.02	4.92
3	0.39	1.87	0.24	1.13	13½	1.75	8.4	1.06	5.11
3¼	0.42	2.02	0.26	1.23	14	1.82	8.71	1.1	5.29
3½	0.45	2.18	0.28	1.32	14½	1.88	9.02	1.14	5.49
3¾	0.49	2.35	0.3	1.42	15	1.94	9.33	1.18	5.67
4	0.52	2.49	0.32	1.51	15½	2.01	9.65	1.22	5.86
4¼	0.55	2.64	0.34	1.61	16	2.07	9.94	1.26	6.06
4½	0.58	2.8	0.35	1.7	16½	2.14	10.27	1.3	6.24
4¾	0.62	2.96	0.37	1.8	17	2.2	10.51	1.34	6.43
5	0.65	3.11	0.39	1.89	17½	2.27	10.87	1.38	6.62
5½	0.71	3.42	0.43	2.09	18	2.33	11.2	1.42	6.81
6	0.78	3.73	0.47	2.27	18½	2.4	11.51	1.46	7.0
6½	0.84	4.05	0.51	2.46	19	2.46	11.82	1.5	7.19
7	0.91	4.35	0.55	2.62	19½	2.53	12.13	1.54	7.38
7½	0.97	4.67	0.59	2.84	20	2.59	12.44	1.58	5.76

Bulk-percentage to be read on the side of the tube.

Estimation of Phosphates.—Proceed as in the estimation of chlorids, except to add 2 c.c. of 50 per cent. acetic acid and 3 c.c. of a 5 per cent. uranium-nitrate solution. The bulk-percentage of uranyl phosphate ($H[U.O_2]PO_4$) is read and calculated in accord with the table for phosphates.

TABLE FOR THE ESTIMATION OF PHOSPHATES AFTER CENTRIFUGATION.

Showing bulk-percentages of uranyl phosphate ($H[UO_2]PO_4$) and the corresponding gravimetric percentages and grains per ounce of phosphoric acid (P_2O_5).—(Purdy.)

BULK-PERCENTAGE OF $H(UO_2)PO_4$.	PERCENTAGE P_2O_5.	GR. PER OZ. P_2O_5.	BULK-PERCENTAGE OF $H(UO_2)PO_4$.	PERCENTAGE P_2O_5.	GR. PER OZ. P_2O_5.
½	0.02	0.1	11	0.14	0.67
1	0.04	0.19	12	0.15	0.72
1½	0.45	0.22	13	0.16	0.77
2	0.05	0.24	14	0.17	0.82
2½	0.055	0.26	15	0.18	0.86
3	0.06	0.29	16	0.19	0.91
3½	0.065	0.31	17	0.2	0.96
4	0.07	0.34	18	0.21	1.06
4½	0.075	0.36	19	0.22	1.06
5	0.08	0.38	20	0.23	1.15
6	0.09	0.43	21	0.24	1.15
7	0.1	0.48	22	0.25	1.25
8	0.11	0.53	23	0.26	1.25
9	0.12	0.58	24	0.27	1.35
10	0.13	0.62	25	0.28	0.35

Bulk-percentage to be read from graduation on the side of the tube.

Estimation of Sulphates.—Proceed as in estimating chlorids, except to add 5 c.c. of barium-chlorid mixture (barium chlorid 4 parts, strong hydrochloric acid 1 part, distilled water 16 parts). The precipitate of barium sulphate ($BaSO_4$) is estimated by the table for sulphates.

TABLE FOR THE ESTIMATION OF SULPHATES AFTER CENTRIFUGATION.

Showing the bulk-percentages of barium sulphate ($BaSO_4$) and the corresponding gravimetric percentages and grains per fluidounce of sulphuric acid (SO_3).—(Purdy.)

BULK-PERCENTAGE OF $BaSO_4$.	PERCENTAGE SO_3.	GR. PER OZ. SO_3.	BULK-PERCENTAGE OF $BaSO_4$.	PERCENTAGE SO_3.	GR. PER OZ. SO_3.
⅛	0.04	0.19	2¼	0.55	2.64
¼	0.07	0.34	2½	0.61	2.93
⅜	0.1	0.48	2¾	0.67	3.22
½	0.13	0.62	3	0.73	3.5
⅝	0.16	0.77	3¼	0.79	3.79
¾	0.19	0.91	3½	0.85	4.08
⅞	0.22	1.06	3¾	0.91	4.37
1	0.25	1.1	4	0.97	4.66
1¼	0.31	1.49	4¼	1.03	4.94
1½	0.37	1.78	4½	1.09	5.23
1¾	0.43	2.06	4¾	1.15	5.52
2	0.49	2.35	5	1.21	5.81

Bulk-percentage to be read from graduation on the side of the tube.

Should the bulk-percentage exceed 15 per cent., the urine should be diluted.

For description of Wetherill's method of torfugation see p. 528.

UREA.

Urea is the most abundant of all the single constituents of normal urine, 20 to 40 gm. (300 to 600 gr.) being excreted by a healthy man of average weight during the twenty-four hours. The greater portion of nitrogen taken with the food is excreted through the urine as urea; therefore retrograde tissue metamorphosis and the unassimilated principles of nitrogenous foods directly influence the elimination of urea.

Urea, $CO(NH_2)_2$, when crystalline, appears as colorless, quadrilateral or hexagonal, silky prisms, with rather oblique ends; or, when rapidly crystallized, as delicate needles. These crystals are permanent in air, but soluble in water. When treated with nitric acid, nitrate of urea ($CON_2H_4HNO_3$) is formed as hexagonal, octahedral, or lozenge-shaped crystals, not freely soluble in water.

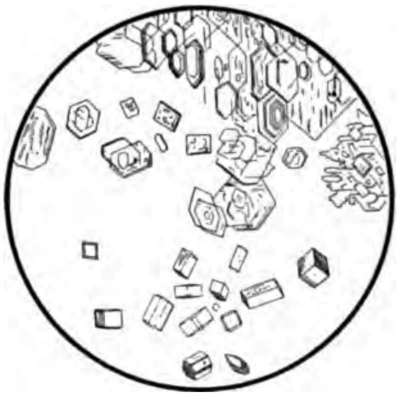

Fig. 77.—Crystals of nitrate of urea (upper half) and oxalate of urea (lower half) (Ogden).

At 100° C. urea gives evidence of decomposition and melts at 130° to 132° C. During decomposition biuret is formed (see *Biuret Reaction*, page 217).

Approximately 85 per cent. of the nitrogen eliminated in the urine is found in the form of urea; the remaining nitrogen consisting of uric acid, kreatinin, hippuric acid, and xanthin bases. It is impossible, however, to estimate the destructive tissue-changes taking place in the body from the quantity of urea eliminated.

The mean amount for healthy men between twenty and forty years is 33.18 gm. (512.4 gr.), and is 0.015 to 0.035 gm. per hour for each kilogram (approximately 2 pounds) of body-weight. Women excrete less urea than men, while children excrete proportionately more than either men or women.

Increase.—An increased elimination of urea is observed in the acute febrile diseases and is due to increased tissue-destruction;—50 gm. are frequently excreted in twenty-four hours both during the fastigium as well as at the time of, and following, the crisis. Such non-febrile conditions as diabetes mellitus, dyspnea, pernicious anemia, scurvy, leukemia, paralysis agitans, minor chorea, epilepsy, gastro-intestinal disorders, functional albuminuria; as well as the following drugs—caffein, ammonium chlorid, potassium chlorid, morphin, codein, and carbonate of lithia—cause an increased excretion of urea. An increase also follows phosphorus-poisoning and the application of electricity.

Decrease.—A decreased elimination of urea is encountered in acute yellow atrophy. It may even be absent and such other nitrogenous bodies as leucin and tyrosin found in its stead. Hepatic cirrhosis and carcinoma, Weil's disease (a peculiar form of infectious jaundice), chronic plumbism, chronic rheumatism, chronic nephritis, osteomalacia, general paresis, melancholia, hysteric outbreaks, catalepsy, Addison's disease, and chronic alcoholism display a decreased elimination, possibly of a nervous character. It has been noted in leprosy and in impetigo. Inhibited hepatic circulation, or excretion and destruction of liver tissue with gastro-intestinal derangements, play important rôles in diminishing the elimination of urea.

Ratio.—The proportionate relation existing between the elimination of urea, phosphates, and chlorids—the latter equaling about one-half that of the urea during health—will often be found of interest.

Quantitative Estimation of Urea.—A number of methods have been devised for the estimation of urea in the urine, which with but few exceptions depend upon its decomposition and the estimation of the nitrogen evolved during this process, or by the difference in density of the urine before decomposition and after it is completed.

Hypobromite Method.—This is by far the most practical method for clinical purposes, despite the moderate degree of error to which it is liable, as it is based upon the decomposition of urea into carbon dioxid and nitrogen when in the presence of sodium hypobromite. The excess of sodium-hydrate solution absorbs the CO_2, leaving only the nitrogen to be estimated.

Reagents.—1. NaOH 100 parts, and H_2O 250 parts.

2. Bromin 1 part, KBr 1 part, H_2O 8 parts. Mix these solutions in proportions of one part of No. 2 to four parts of No. 1.

The hypobromite solution (dissolve 100 gm. of caustic soda in 250 c.c. of water and permit to stand until it is cool, when 25 c.c. of bromin are added) if placed in amber or green glass-stoppered bottles and kept in a cool place, will keep for a fortnight. Many writers prefer a much weaker solution; while others again have two solutions, and mix them together when needed. It is with this latter method that I have obtained the most satisfactory results.

A number of forms of apparatus have been devised, but the one which appears to be most convenient is that lately recommended by Doremus (Figs. 78, 79).

Procedure.—1. A small amount of urine is placed in tube (*c*) —stop-cock (*b*) closed—and the stop-cock is opened for a moment and then closed in order to fill its lumen.

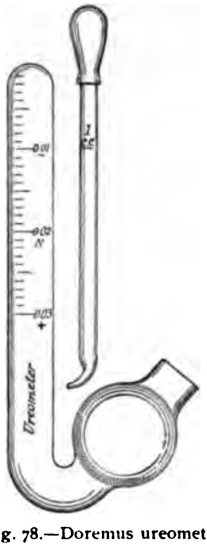

Fig. 78.—Doremus ureometer.

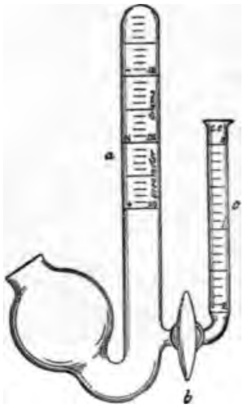

Fig. 79.—Hinds' modification of the Doremus ureometer.

2. Notice that both tubes are now free from urine, and fill tube (*a*) with the hypobromite solution.
3. Next place the urine in tube (*c*).
4. The stop-cock is now turned slightly and the urine is allowed to mingle slowly with the hypobromite solution until one-half or one c.c. has passed from (*c*) to (*a*). The nitrogen generated is collected at the top of (*a*). The degrees graduated indicate the amount of urea (in grams or grains) in the quantity of urine employed.

Caution.—Should the urine be concentrated, a dilution of one part of urine in 4 or even in 10 parts of water will give more constant readings, which are to be multiplied by the amount of dilution used. Again, thermic fluctuations are known to influence the reading slightly, but for clinical purposes this is not worthy of consideration.

Estimation of Total Nitrogen.—The method suggested by Kjeldahl will serve as one convenient for this purpose; the first step being to decompose the organic matter by means of sulphuric acid, when all nitrogen not in combination with oxygen is converted into ammonia. An excess of sodium hydrate is now added and distilled, received into a known quantity of titrated acid, and the excess again titrated with sodium hydrate; thus ascertaining the amount of ammonia requisite. Since 17 gm. of ammonia correspond to 14 gm. of nitrogen, the quantity of nitrogen is readily ascertained.

Reagents.—1. Gunning's mixture—15 c.c. concentrated sulphuric acid; 10 gm. potassium sulphate; 0.5 gm. copper sulphate.

2. Solution sodium hydrate (270 gm. to 1000 c.c., specific gravity, 1.243).

3. Pulverized talcum or granulated zinc.

4. A one-fourth normal solution sulphuric acid.

5. A one-fourth normal solution sodium hydrate.

Application.—1. Ten c.c. of urine are placed in a flask and treated with reagent (1). The flask is placed at an angle of 45 degrees and heated just to the boiling-point, until the solution is clear.

2. Cool and transfer the contents of the flask to a retort with the aid of water, and treat with a slight excess of sodium hydrate solution (5) (40 c.c. of which will equal 5 c.c. of sulphuric acid).

3. Add a small amount of talcum or granulated zinc (3), connect retort with the condenser, and begin distillation, continuing until two-thirds of the solution has passed over. The nitrogen bulb into which the distillate is received should contain a definite quantity of a one-fourth normal solution of sulphuric acid (4), of which 25 to 35 c.c. are usually required. Disconnect the condenser whenever the distillation is completed, and wash it with distilled water, adding the washing to the distillate.

4. Add a few drops of tincture of cochineal or phenolphthalein.

5. Titrate the excess of sulphuric acid with the one-fourth normal solution of sodium hydrate (5), and the quantity found to be present is deducted from the 30 c.c. employed. Continue the titration until the yellow is replaced by a rose color. The difference multiplied by 0.0035 equals the amount of nitrogen present in the 10 c.c. of urine; but if, instead, the figure representing this difference be multiplied by 20, it is equivalent to the amount of urea present in the 10 c.c. of urine.

URIC ACID.

Uric acid results from the decomposition of a certain variety of albuminous substance—nucleins. The nuclear nucleins (containing a nucleinic acid radicle) appear to constitute the mother substance of uric acid. Paranucleins are known to be deficient in this particular, and they in no way influence the elimination of uric acid.

The daily elimination for normal man is found to be between 0.2 and 0.5 gm. Kossel has demonstrated forms of nucleinic acid which are known as the xanthin, alloxur, or purin bases, and, according to Fischer, are probably derived from purin. Monomethyl-xanthin or heteroxanthin, dimethyl-xanthin or paraxanthin, trimethyl-xanthin, as well as theophyllin and theobromin are also closely allied to this group.

Uric acid of the urine may be found to occur as a compound in which one molecule of sodium is combined with two molecules of uric acid. The conditions affecting its solubility are the quantity of water, presence of inorganic salts, and the reaction of the urine. Uric acid is precipitated from urines containing an excess of disodic phosphate. Such basic substances as sodium, ammonium, and potassium are known to occur with uric acid, and when these salts are decomposed by strong acids, uric acid is set free. Uric acid in its purity occurs as colorless rhombic plates, but that which precipitates from the urine is oftenest of a reddish-brown hue (see *Microscopy*, page 264).

Increase.—After the ingestion of such foods as liver, kidney, thymus gland, and brain,—rich in either the purin bases or in nuclear nucleins,—an increased elimination occurs. It also takes place after excessive cell destruction of the body tissues, especially the leukocytes, five hours after a full meal; during and immediately after a paroxysm of gout; in acute articular rheumatism with high temperature; and in leukemia the elimination is increased. In the latter condition it has been known to reach 5 gm. per diem.

Decrease.—A decreased elimination may be noticed as the result of a purely vegetable diet; preceding an attack of gout; during the course of diabetes, chronic plumbism, chronic interstitial nephritis, pseudo-hypertrophic paralysis, progressive muscular atrophy, and in chlorosis. In the secondary and pernicious types of anemia decreased elimination is also a prominent feature.

Tests for Uric Acid.—**Murexid Test.**—1. Place a few crystals of uric acid on a porcelain dish, add 5 to 10 drops of concentrated nitric acid, and heat sufficiently to evaporate the acid. A yellowish-red color remains upon the porcelain.

2. Cool, and add a drop of ammonia, when a purplish-red color develops (murexid).

3. A drop of sodium hydrate causes this to change to a reddish-blue color which disappears on heating.

Copper Test.—1. Dissolve crystals of uric acid in a solution of sodium hydrate.

2. Treat the mixture with Fehling's solution and heat, when a white precipitate will develop (white urate of copper), but should sufficient Cu sulphate be present, a reddish precipitate appears (cuprous oxid).

Carbonate-of-silver Test.—1. Dissolve crystals of uric acid in a solution of sodium or potassium carbonate.

2. Place a few drops on a paper, and to it add a drop or two of a solution of nitrate of silver, when a distinct gray stain occurs. Urates also cause this reaction.

Quantitative Estimation of Uric Acid.—*Reagents.*—Dissolve 1.58 gm. of potassium permanganate in one liter of water (1 c.c. of this solution is decolorized by 0.00375 gm. of uric acid). Place 100 c.c. of clear filtered urine into a beaker, and to it add 35 gm. of pure ammonium chlorid; stir well, and to the mixture add ammonia and water to give it an alkaline reaction. Agitate the fluid by stirring for five minutes, then allow it to stand for five minutes, by which time a precipitate will have settled near the bottom of the beaker. This precipitate is composed of earthy phosphates together with the whole of the uric acid as hydrogen ammonium urates. Filter and wash with a saturated solution of ammonium chlorid the precipitate which collects upon the filter. Remove the funnel and wash the precipitate into a beaker by means of a jet of water from the wash-bottle. The funnel is returned to its support, and underneath it is placed the beaker into which the precipitate has been washed. Hot water containing a trifle of sodium carbonate is now poured through the filter. Repeat this process three times, heat the beaker and its contents, and then add 10 c.c. of concentrated sulphuric acid. A decidedly acid reaction is produced. Immediately titrate with a standard solution of potassium permanganate, and the percentage of uric acid in the urine is estimated from the number of cubic centimeters of permanganate solution employed.

Reaction.—A weak solution of potassium permanganate displays a decided rose color which it loses instantly when allowed to flow slowly into the hot solution of uric acid in dilute sulphuric acid, shaking after each addition of the permanganate solution. This disappearance of the rose coloring of the permanganate solution is caused by the uric acid, which rapidly decomposes the potassium permanganate. Whenever a single drop in excess of the potassium permanganate is added, its rose color persists and *the end point of the titration is thus placarded.* Titration must be carried out at a temperature between 60° and 80° C.

Method of Computation.—The percentage of uric acid present in the given specimen of urine is determined in the following manner:

0.00375 × No. c.c. of permanganate solution employed. In every 100 c.c. of urine 0.001 gm. of uric acid escapes precipitation, therefore it requires this addition to the final product. The results obtained by this method, while sufficiently reliable for general clinical work, should not be compared with those obtained by the more complicated methods.

Ludwig-Salkowski's Method.—This method is based upon the principle that a solution of uric acid in sodium carbonate treated with a solution of nitrate of silver—an excess of ammonia having previously been added to the mixture—induces a flaky gelatinous precipitate composed of uric acid, silver, and sodium; this precipitate being only feebly, if at all, soluble. The silver may be removed, leaving compounds of uric acid and sodium capable of decomposition by the addition of hydrochloric acid.

Precautions.—1. Concentrated urines should be diluted one-half.

2. The specific gravity should be nearly 1.020, and urines of a lower specific gravity should be evaporated to approximate this density.

3. When there is a distinct deposit of uric acid at the bottom of the urine to be examined, this too should be collected and estimated.

4. Albumins should be removed from the urine.

5. In case the urine contains sugar, from 500 to 1000 c.c. are treated with a neutral solution of acetate of lead; this is filtered, and the filtrate is then precipitated with mercuric acetate. This precipitate consists mostly of mercuric urates, and after having stood for twenty-four hours, should be separated by filtration. Wash and suspend in water. Remove the mercury by means of sulphureted hydrogen, filter off the sulphid of mercury, and preserve the filtrate. Thoroughly boil the precipitate with water, again filter, and the washing obtained is added to the filtrate previously preserved. Evaporate the total quantity of fluid to a rather small volume; acidify with hydrochloric acid, when the uric acid separates out and may be treated by one of the methods previously named.

6. It is essential that rapidity be exercised while conducting the various steps in Ludwig-Salkowski's method.

Method of Application.—*Reagents.*—1. Ammoniacal magnesium mixture. Dissolve 100 gm. of crystallized magnesium chlorid in a sufficient quantity of water, and to it add (cold) a saturated solution of magnesium chlorid in excess, and sufficient strong ammonium to lend a decided odor to the mixture. Where the mixture remains cloudy add more ammonium-chlorid solution. Dilute to a liter and place in a glass-stoppered bottle.

2. Dissolve 26 gm. nitrate of silver in distilled water, and then add ammonia sufficient to dissolve the brown precipitate. Dilute to a liter and place in a colored, glass-stoppered bottle.

Place in a vessel 250 c.c. of filtered urine and treat with 50 c.c. of ammoniacal magnesium mixture to remove the phosphates. Filter immediately, thereby preventing the formation of a small amount of magnesium urate. Place 250 c.c. of the filtrate, which corresponds to 200 c.c. of the urine, in a vessel and treat with a solution of nitrate of silver (3 per cent.). Should the precipitated silver chlorid, which forms soon after the addition of the silver, disappear on stirring, add a few drops of ammonium hydrate. A flaky precipitate now separates and is allowed to settle. Acidify a few cubic centimeters of the supernatant fluid with nitric acid to determine whether or not enough of the silver-nitrate solution has been added, and in the event of a distinct clouding, suggestive of silver chlorid, sufficient of the silver solution has been added. Should this clouding not appear, the mixture needs to be rendered alkaline with ammonium, returned in the beaker, and again treated with the silver solution. Filter rapidly through a rather loose paper, all precipitates being removed from the beaker by means of a glass rod. Wash the precipitate until the specimen of the washing is not rendered turbid by nitric acid, and until only a slight turbidity follows the addition of a drop of silver solution. The precipitate and the filter are placed in a wide-mouthed flask which contains about 200 c.c. of distilled water. Agitate vigorously. Pass sulphureted hydrogen through the mixture, bring to the boiling-point, and render distinctly acid by adding a few drops of hydrochloric acid; at which time the sulphid of silver and the paper are rapidly separated by filtering. Should this separation be effected slowly, there is liable to be an admixture of silver to the uric acid. Wash the contents of the filter several times with hot water, and quickly evaporate both the filtrate and the washings to but a few cubic centimeters. Add a few drops of hydrochloric acid and allow to stand in a cool place for twenty-four hours. When the mixture contains silver there is a cloud following the addition of the hydrochloric acid. The dried uric acid needs to be washed with carbon disulphid. Collect the uric acid that has separated from the solution on a dried and weighed filter. Wash with water, then with 90 per cent. alcohol, and finally with absolute alcohol and ether. The water employed for washing should be collected separately, and for every 20 c.c. used 0.0048 gm. should be added to the weight of the uric acid obtained.

XANTHIN BASES.

Collectively the substances comprising the xanthin bases and uric acid are referred to as the alloxur bases and the purin bases. The xanthin bases of the urine are xanthin, hypoxanthin, heteroxanthin, paraxanthin, adenin, and guanin; and unlike uric acid, xanthin bases are to be found in both animal and vegetable tissues. Under normal conditions the urine will be found to contain a very small percentage of xanthin, amounting in bulk to about 10 per cent. of the uric acid. As a rule, it is increased in amount when the uric acid shows a decided increase. At times, however, there is an increase in the uric acid together with a diminution in the xanthin bases; and less frequently the reverse condition is met. It is to be remembered that uric acid is an oxidation product of the xanthin bases, and that its origin is the same, which serves to explain their varying proportionate relations. Our present knowledge of the xanthin bases and their relation to pathologic states is of but limited clinical value. The normal quantity is said to fluctuate between 0.0286 and 0.0561 gm. per diem. Paraxanthin and heteroxanthin occur but sparingly in the urine.

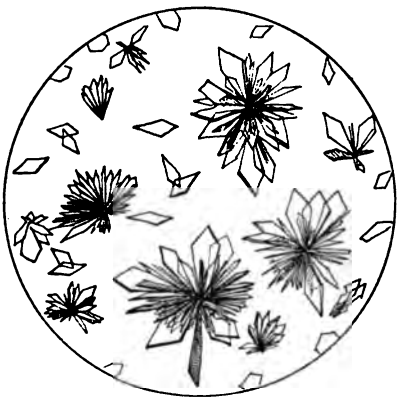

Fig. 80.—Xanthin crystals (after the drawings of Horbaczewski, as represented in Neubauer and Vogel).

Clinical Significance.—The xanthin bases are increased in croupous pneumonia, leukemia, nephritis, and after a meal containing an abundance of nucleins.

Tests.—(1) Lift a small portion of the urinary sediment into a pipet and transfer it to a porcelain dish; (2) add a few drops of nitric acid and evaporate to dryness. Should xanthin be present, the residue is yellow, but upon the addition of a few drops of sodium-hydrate solution and the application of heat, a red color develops. This reaction is common to the xanthin bases.

Quantitative Estimation.—1. **Salkowski's method** consists in precipitating 600 c.c. of the filtered urine with 200 c.c. of magnesium mixture (see *Ludwig-Salkowski's method,* page 198), after which add a solution of nitrate of silver (3 per cent.) to 700 c.c. of the filtrate in a proportion of 6 c.c. per hundred, observing all precautions set forth in Ludwig-Salkowski's method for uric acid. 2. Allow the mixture to stand for an hour, filter, and wash the precipitate in water until all the free silver has been removed. 3. Perforate the filter and wash the precipitate into a flask with 600 to 800 c.c. of water that has been acidified with hydrochloric acid, and decompose with sulphureted hydrogen. 4. Heat over a water-bath to remove any excess of sulphureted hydrogen; filter to separate the sulphid of silver and evaporate the filtrate to dryness. 5. Treat the residue with 30 c.c. of sulphuric acid (1-100); boil the solution, and then allow it to stand for twelve hours. 6. The uric acid will be found to have separated from the liquid, which should be filtered, washed with a small amount of dilute sulphuric acid, and later with alcohol and ether, and weighed. Add 0.0005 gm. to the result for every 10 c.c. of the acid filtrate employed—allowing for the trace of uric acid thus lost.

7. After the uric acid has been removed by filtration, the filtrate is treated a second time with ammonium and silver solution to precipitate the xanthin bases. 8. Collect this precipitate on a filter, wash with water, dry, and incinerate. 9. Dissolve the ash in nitric acid and the silver is estimated by titration with a solution of potassium sulphocyanid, using ammonioferric alum (saturated solution at ordinary temperature) as an indicator. The solution of potassium sulphocyanid described in the estimation of the urinary chlorids (page 180) may be employed, and this solution is of such strength that 1 c.c. corresponds to 0.00734 gm. of silver. One atom of silver when in combination with silver compounds of xanthin, guanin, etc., represents 0.277 gm. of nitrogen or 0.7381 gm. of alloxur bases. One c.c. of the potassium sulphocyanid solution represents 0.002 gm. of nitrogen and likewise 0.00542 gm. of alloxur bases.

HIPPURIC ACID.

Under normal conditions from 0.1 to 1 gm. of hippuric acid is excreted through the urine during every twenty-four hours. This is probably derived largely from albuminous material. The elimination continues during the administration of albuminous materials as well as during starvation. It usually occurs in combination with potassium, magnesium, sodium, and calcium; and is readily soluble in solutions of the alkaline hydrates and carbonates, alcohol, and ether. Its salts thus formed are decomposed by strong acids, in which instance hippuric acid separates out and may be collected and weighed.

Quantitative Estimation.—Simon's Method.—1. Evaporate over a water-bath 500 c.c. of fresh urine to a syrupy consistence, keeping the urine neutral in reaction by the addition of sodium carbonate during the entire process of evaporation. 2. Extract the residue with cold alcohol, 90 to 95 per cent., employing a quantity about half that of the urine. 3. Stand the mixture aside for twenty-four hours. 4. The alcoholic filtrate will be found to contain the salts of hippuric acid, and these are liberated from the alcohol by distillation. 5. Acidify the remaining solution with acetic acid, and extract with five times its own volume of alcoholic ether (alcohol 1 part; ether 9 parts). 6. From the combined extracts the ether is distilled off and the remaining solution evaporated over a water-bath. 7. Boil the residue with water, allow it to cool, and pass through a well-moistened filter. Hippuric acid is soluble in boiling water, and is in this way separated from other constituents soluble in alcohol and ether. 8. Render the filtrate alkaline with milk of lime, removing all excess of calcium by passing carbon dioxid through the mixture, which is later boiled and filtered. 9. Shake with ether to remove all impurities, and any calcium salts remaining in solution are decomposed by acid, and the solution extracted with ether. 10. Evaporate this remaining solution to but a few cubic centimeters, and upon standing hippuric acid will separate. 11. Dry the crystals upon plaster-of-Paris plates, shake with benzol and petroleum-ether for the purpose of removing any benzoic acid that may be present, and weigh. These crystals of hippuric acid may be recognized under the microscope and also by their solubility, etc.

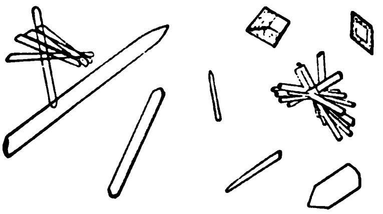

Fig. 81.—Hippuric-acid crystals (Ogden).

OXALIC ACID.

The oxalic acid in the normal urine is probably derived from two sources. One of these is a vegetable diet, while the other doubtless exists in the body, though its manner of action is not thoroughly understood. This latter variety may be derived from the uric acid through oxidation, since oxaluric acid is capable of being produced from uric acid, and oxaluric acid is also readily decomposed into oxalic acid and urea. Again, oxalic acid may result from the imperfect oxidation of carbohydrates.

Excess.—The excretion of oxalic acid through the urine becomes of special pathologic importance since many vegetables are capable of causing an excessive elimination. Among such vegetables are spinach, carrots, tomatoes, string-beans, celery, and asparagus; and also grapes and apples. Gastro-intestinal derangements are liable to cause an increased excretion of oxalic acid, which is doubtless the result of incomplete digestion and oxidation of carbohydrates. Nervous oxaluria may result in a similar manner.

Transitory albuminuria has been found to be associated with oxaluria (Senator), and the late J. M. DaCosta has outlined not unlike conditions. In connection with diabetes there are to be seen peculiar relations between the uric acid, carbohydrates, phosphates, and oxalic acid excreted.

Oxalic-acid Diathesis.—In this condition there is a decidedly increased production of oxalic acid accompanied with a temporary retention which of necessity must later be followed by an increased elimination. This condition does not appear to be materially influenced by diet, and may result from abnormal metabolic processes within the body tissues. It has been suggested that this peculiar production of oxalic acid is not unlike that interested in the production of diabetes mellitus, and while it is impossible at present to point out clearly the relation existing between these two conditions, it is doubtless one deserving of further physiologic research.

The quantity of oxalic acid normally excreted during twenty-four hours varies from that of a mere trace to 20 mgm. (0.31 gr.). Oxalic acid is readily detected in the urine by its characteristic crystals (Fig. 95), though it is impossible to determine with any degree of certainty the amount of oxalic acid excreted from the number of crystals of calcium oxalate present upon microscopic examination.

Again, urines rich in calcium oxalate may display no crystals of this substance even after standing for several hours. It is my practice to neutralize cautiously such urines by adding a few drops of ammonia, when it is often possible to induce crystallization of the salt, and should this procedure be ineffectual, the following method must be employed:

Quantitative Estimation of Oxalic Acid.—1. Treat 600 c.c. of fresh urine with a small quantity of an alcoholic solution of thymol—in this way preventing putrefaction—and then treat it with calcium chlorid and ammonia, adding these substances in excess. 2. Diacid sodium phosphate, which holds the oxalic acid suspended in solution, is in this way removed, and the precipitate is treated with just sufficient acetic acid to dissolve it. 3. The calcium oxalate being insoluble in acetic acid is gradually precipitated, when the mixture is allowed to stand for twenty-four hours. 4. The calcium oxalate is now separated by means of a filter; washed with a small amount of water and dissolved with a few drops of hydrochloric acid. 5. Add sufficient dilute ammonia to the filtrate to give the solution a feebly alkaline reaction; allow to stand for twenty-four hours, when the calcium oxalate will have separated from the solution and may be collected upon a filter the weight of whose ash is known.

Wash with water the contents of the filter, dry, and incinerate in a crucible, heating vigorously for at least twenty minutes, thus transforming the oxalate into oxid. The ash is weighed and the corresponding amount of oxalic acid calculated. By weight, 56 parts of calcium oxid are equivalent to 128 of calcium oxalate; therefore $56 : 128 :: y : x$—x being equivalent to $2.2857\,y$ in which y is indicative of the quantity of calcium oxid recovered from a known quantity of urine. The quantity of calcium oxalate is represented by x.

KREATIN AND KREATININ.

The former of these substances is constantly present in muscle tissue, and is in all probability the immediate and perhaps constant antecedent of kreatinin, which forces one to consider at least two sources of this body when found in the urine, namely, the body muscular tissue and the ingestion of muscle tissue as food. Nearly 1 gm. of kreatinin represents the daily excretion through the urine of a healthy adult. Thus far the pathologic variations in the excretion of kreatinin have failed to be of clinical value. The increased destruction of body tissue which takes place during the course of such febrile conditions as pneumonia and typhoid fever may be placarded by an increased elimination of kreatinin in the urine. Such patients are not upon a meat diet; yet parallels have not been drawn between the excretion of urinary kreatinin under such conditions and the destructive changes in the body tissues.

Weyl's Test.—Treat 10 to 20 c.c. of the urine with a few drops of a dilute solution of sodium nitroprussid, and then add to the mixture, drop by drop, a weak solution of sodium hydrate. Should kreatinin be present in appreciable amounts, the urine becomes a ruby-red color; this change being more conspicuous at the bottom of the liquid. The red color is but temporary and gives way in a few minutes to an intense yellow. Through the application of heat and the addition of a few drops of glacial acetic acid in pure solution, the yellow color is replaced by a green.

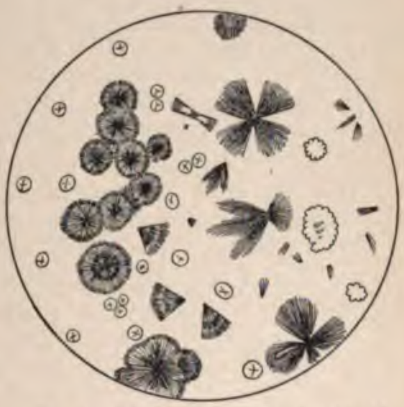

Fig. 82.—Crystals of kreatinin-zinc chlorid (Salkowski).

PROTEIDS IN THE URINE.

Any one, or in fact all, of the proteids of blood plasma may appear in the urine, namely, serum-albumin, serum-globulin, and fibrinogen. In addition to these it is not uncommon to meet with compound proteids—mucin and nucleo-albumin (normal constituents of the urine), and with the albumoses, among which Bence-Jones' albumose should be given special mention. It is questionable whether true peptone occurs in the urine. Rare, indeed, it is that during health any of the pathologic proteids escape through the urine.

Mucin.—Mucin is to be found in normal urine, though but a trace may be present; and it is the chief constituent of urine containing a large quantity of mucus. Mucin is derived from the muciparous glands communicating with the urinary tract.

Increased.—A decided increase is to be observed in connection with a catarrhal condition of the urinary tract, when, on standing, it collects at the bottom of the urine in the form of a rather heavy, creamy, loose, viscid, slimy, tenacious precipitate.

Detection.—1. Mucin is precipitated from the urine by vegetable acids, such a precipitate being insoluble in an excess of the acid employed for its precipitation. (Where the urine contains a liberal amount of albumin, first remove this body by boiling and filtering.) 2. Add to the precipitate three times its volume of strong alcohol, and set aside for several hours. 3. Filter, wash the precipitate with alcohol, and then with warm water. 4. The filtrate which contains the mucin is now acidified with acetic acid, and should a turbidity appear, mucin is present.

SERUM-ALBUMIN AND SERUM-GLOBULIN IN THE URINE.

These substances may occur in the urine either singly or together, the latter condition being far more common and constituting what is ordinarily termed "albuminuria." Albuminuria may result from disease of the kidneys, or from acute or chronic congestion, etc. The use of drugs, including ether and alcohol; the use of albuminous foods; high fever (febrile albuminuria), exertion, cold baths (functional albuminuria), and standing posture (orthostatic albuminuria) may be followed by albuminuria.

Albumin may originate from congestion or ulcerations of the bladder, urethra, vagina or uterus. It follows reduced blood pressure and occurs late in the anemias. Albuminuria may be coexistent with oxaluria, phosphaturia or with diabetes. Accidental albuminuria results from an admixture of blood or pus.

Separation of Albumin and Globulin.—(1) Place a few cubic centimeters of urine in a beaker, render it slightly alkaline with ammonia, filter to remove the phosphates, and then to 100 c.c. of the filtrate add its own volume of a saturated neutral solution of ammonium sulphate, allowing it to stand for an hour; by this time the globulin and probably a portion of the mucin will have separated from the solution. (2) Collect this precipitate on a weighed, ash-free, dried filter, and wash with a solution of ammonium sulphate, 50 per cent. saturation, after which dry and weigh. (3) After drying, incinerate, and detect the weight of the ash, the difference being equal to the amount of globulin present.

The total proteids may be estimated by complete saturation of the urine with ammonium sulphate, and drying the precipitate in the above manner. The difference between the total proteids and the globulin is reckoned as albumin.

Douglas' Method.—The method which I have adopted quite generally for the recognition of albumin, and one which appears to have received the sanction of foreign authors (though not now popular in America), is the contact method for the detection of serum-albumin. Carstairs Douglas * has carefully determined the quantity of albumin necessary in a given urine to produce the characteristic ring upon contact with nitric acid. He further studied a series of dilutions made from urines, the percentage of albumin contained in such specimens having been previously ascertained, and concludes that 0.0033 per cent. of albumin is sufficient to cause the ring to appear in from two to three minutes. For example, a urine after dilution ten times showed a ring as above outlined. Multiply 0.0033 by 10 = 0.033 per cent. of albumin, and in a like manner higher dilutions were employed with equally satisfactory results.

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* "Chemical and Microscopical Aids to Clinical Diagnosis," 1899, p. 70.

In my experience this method has not served so well as the estimation of the total quantity of albumin in the urine by the Esbach method (page 211); yet for the average daily estimation it is probably sufficiently near to accuracy to enable one to determine with safety the amount of albumin as being increased or decreased in a given instance. I have been able to obtain better results by applying the contact method, using nitric acid, in the pipet (see *Author's Method*).

Precaution.—In employing Douglas' method the urine should be diluted with great care by means of a buret, and at least one inch of nitric acid should be placed either in the test-tube or allowed to enter the tube (author's method), when the diluted urine is brought in contact with it and allowed to occupy a space above the acid. The time necessary for coagulation to produce the ring is also important, three minutes being the limit. The albumin ring is rendered conspicuous by holding the glass or pipet containing the urine in front of a dark background. Among the various reagents suggested by as many writers I have found but two that are capable of withstanding the tests necessary to render them worthy of special clinical mention; namely, concentrated nitric acid, and the nitromagnesium mixture (Roberts' solution), which is composed of:

Pure concentrated nitric acid	2 parts	
Saturated solution magnesium sulphate	10 "	M.

The application of these substances as originally outlined by Heller was to place a few cubic centimeters of the reagent in a test-tube and allow the urine to flow slowly from a buret into the tube, where it formed a layer above the reagent.

Author's Method.—After employing Heller's method for some years, the following method of application suggested itself to me, and its employment has since been attended with most satisfactory results.* Albumin causes a white cloud to appear in the form of a ring at the zone of contact of the two liquids (reagent and urine), and this test when carefully applied must be regarded as one of great value.

1. A pipet is filled for a distance of from one inch to one and

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* "N. Y. Med. Jour.," May 24, 1902, p. 885.

one-half inches with urine to be tested. Then this pipet is either carried under a stream of water and dried by a towel or all the urine is removed from its surface by a damp towel.

2. This pipet, with its contained urine, is placed near the bottom of a bottle containing pure nitric acid, when the pressure of the index-finger is lessened and the acid allowed to flow gradually up into the pipet (Fig. 83).

3. When the pipet is seen to contain about the same amount of the acid and of the urine, the finger is again pressed firmly upon the top of the pipet, which is then removed from the bottle and held toward the light on a level with the eye; when, if albumin be present, a distinct white cloud in the form of a ring appears at the zone of junction of the urine and the reagent (Fig. 84). The ring is often intensified by placing the pipet in different lights or against a dark background. The hand when placed back of the pipet and carried slowly above and then below the level of the ring serves this purpose.

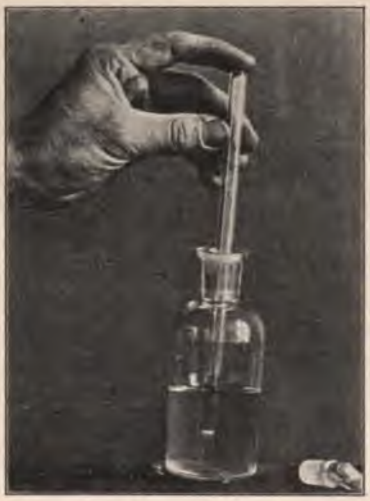

Fig. 83.—Method of filling lower portion of pipet with nitric acid.

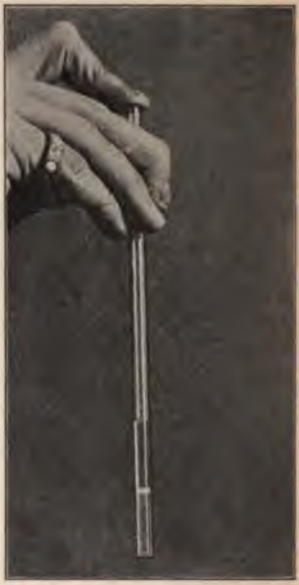

Fig. 84.—Pipet containing an upper stratum of urine, a lower stratum of nitromagnesium solution, and showing a white line (albumin ring) at zone of contact.

The time occupied in performing this test is very slight, usually only a few seconds. Its ease of execution, the fact that the albumin ring is not affected by the jarring of the pipet due to it being formed in a tube of small caliber, the small quantity of both urine and reagent needed, and the fact that it does away with the use of test-tubes, render this method of extreme practical value to both the general practitioner and the laboratory worker.

At times it may be preferable to apply the nitric acid with the addition of heat, in which case the acid is placed in a test-tube and heated to the desired temperature; then a pipet containing the urine to be tested, as before described, is carried to the bottom of the tube and the acid allowed to slowly enter the pipet. The pressure of the index-finger should not be entirely removed until the acid has forced the column of urine up into the pipet, so as to make its superior surface on a plane above that of the acid occupying the space between the outer surface of the test-tube and the pipet (Fig. 85).

By this method the urine and the reagent may be set aside and examined at intervals. The ring is not affected by returning the finger pressure at the top of the pipet and carrying the reacting specimen about the room for class demonstration. Through the courtesy of Prof. George H. Meeker, of the Medico-Chirurgical College, I have been provided with a testing pipet which serves well in this capacity. This method of keeping the nitric acid in contact with the urine for several hours is of special value in the estimation of uric acid, but it is necessary that the column of urine extend for fully two and one-half inches above the zone of contact. The ring caused by an excess of uric acid is less compact than the one produced by albumin, and always occurs high above the zone of contact.

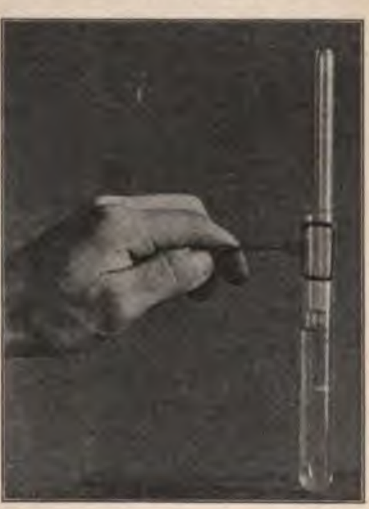

Fig. 85.—Test-tube containing hot nitric acid, and pipet containing albuminous urine. White line (albumin ring) at zone of contact.

Appearance of the Reaction Ring and its Significance.—Here it is my object to elucidate as nearly as possible the value of the reaction obtained by the analysis of urines containing certain named substances; and an important fact in this connection is not to overestimate many of the less common of these reactions.

It is ever to be borne in mind that more than one substance capable of producing color and other changes may be present in the same urine, thereby confusing the results. The ring due to serum-albumin and other albuminoid bodies is fairly constant in its general characteristics, and comprises what the writer has been forced to accept, after five years of daily application, as a most trustworthy test.

Normal Urinary Pigments.—In highly colored urines a reaction may be detected at or near the zone of contact, which produces a more or less distinct cloud, varying from a pink tint to a dull brick-red color.

Serum-albumin.—When this body is present in quantities of pathologic moment the ring is seen at the zone of contact as a whitish band, the thickness of which varies in different specimens. Such variations are influenced (1) by allowing the acid to enter the pipet too rapidly, and (2) by the amount of albumin present in the specimen under observation.

Globulin, albumoses, and peptones may also cause a small ring at the zone of contact. In the aggregate it may be said that the ring caused by these bodies is less clearly outlined by distinct margins characteristic of serum-albumin (see ***Albumose*** and ***Peptone***, pages 216, 217). The cloud of albumin may at times be slightly colored by the pigments present in the urine. This feature is most notable in connection with biliuria and hematuria.

Mucin (Nucleo-albumin).—When present in sufficient amounts this produces a ring which resembles that of serum-albumin in color, but never presents a clear, pearly white tint, and, on the whole, this ring is less perfect than is that of serum-albumin even when of the same thickness; and, in striking contrast to albumin, it appears some distance above the zone of contact.

Urates.—Urates may cause a distinct ring, varying in intensity from a slight reddish tinge to a deep cherry or brownish red. This ring occurs very near, but above, the zone of contact, and often requires close inspection to detect the thin zone of urine between the ring and the superior surface of the reagent. In some specimens the ring forms from one-eighth to one-half inch above the zone of contact.

Biliuria.—Bile-pigments, when present in considerable amounts, cause a decided play of colors at the zone of contact. The ring of serum-albumin in such urines is apt to be colored, as is also the ring produced by mucin. After all known methods are employed to remove these two bodies (albumin and mucin) from the urines, a decided reaction-ring still persists about one-quarter to one-half inch above the zone of contact. From these results it would appear that nitric acid and the other contact reagents have not proved to be reliable tests for serum-albumin when in connection with bile-pigments.

Indican.—Here, too, color changes are observed, which assume a violet hue and vary in intensity with the amount of indican present. The change is seen slightly above the zone of contact.

Resinous Bodies.—These are usually precipitated by nitric acid, but seldom confuse the reaction for albumin. A further discourse by the author upon reagents may be found in the "New York Medical Journal," May 24, 1902.

Quantitative Estimation of Albumin.—**Heat.**—Comparative results are to be obtained by boiling a given quantity of the urine which is acidified with acetic acid; and allow to stand for twenty-four hours in order that the albumin may collect at the bottom of the tube. A graduated tube may be employed for this purpose, but the ordinary test-tube gives fair results. The tube and its contents obtained from the first boiling may be corked tightly and set aside to be used for a comparison with the amount of precipitate that results from future tests; although a far better plan is to record the depth of sediment present at each examination. The albumin may at times separate out into large flakes, again into small flakes, and this in conjunction with other appreciable conditions causes the quantity of albumin to fluctuate greatly when estimated by this method. (It can be recommended, however, to the general practitioner.)

Volumetric Method.—This is by far the easiest method of execution and, therefore, deserves special mention. Take 10 to 20 c.c. of the urine and to it add sufficient distilled water to make the total reach 50 c.c. Treat with two drops of a 1 per cent. aqueous solution of true yellow, and later titrate with a 25 per cent. solution of salicyl-sulphonic acid until the mixture assumes a brick-red color. Multiply the number of cubic centimeters of reagent employed by 0.01006, which will indicate the amount of albumin in the number of cubic centimeters of urine examined. Urine employed for this test should be acid in reaction. When alkaline, acidify with acetic acid.

Esbach's Method.—This method for the estimation of the quantity of albumin is subject to rather decided variations and even liable to gross error; yet if all precautions herein outlined are observed, it will be found the most practical method now in vogue for the quantitative estimation of albumin.

Reagents.—

Picric acid	10 gm.
Citric acid	20 "
Distilled water	1000 c.c.

A special tube (Fig. 86) is to be employed, and upon it will be found the letter U, indicating the portion of the tube to be filled with urine, and nearer the top of the tube is seen the letter R, which indicates the level to which the reagent is to be added. Beginning at the bottom of the tube is a scale graduated from 1 to 7.

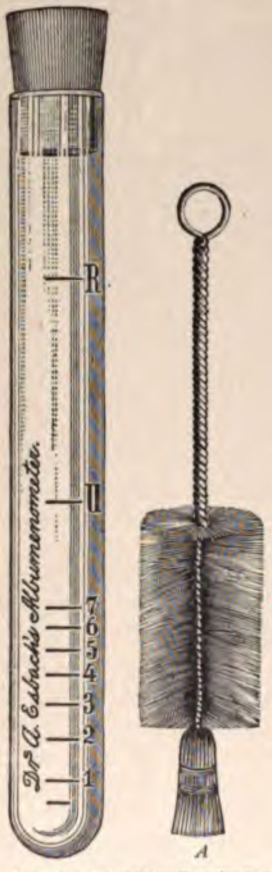

Fig. 86.—Esbach's albuminometer: *A* represents the author's brush for cleansing the albuminometer. In the actual brush the handle is about nine inches long.

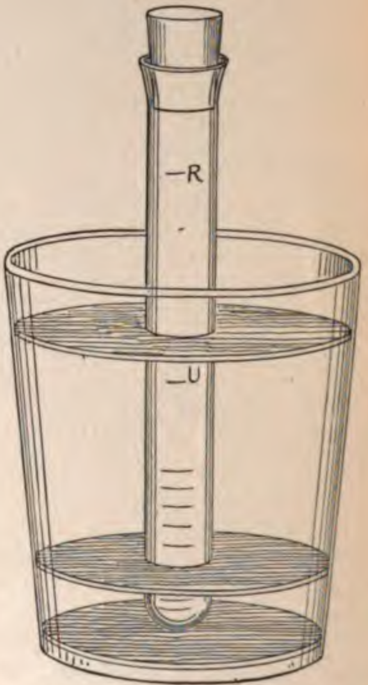

Fig. 87.—Receptacle for Esbach's albuminometer, devised by Dr. W. G. Mudie while working in the author's laboratory. In the bottom of an ordinary tumbler place a piece of heavy cardboard and cut central openings in two other cardboards which are glued in position.

Process.—Fill the Esbach tube with filtered acid urine to U, and add the reagent until it reaches R. Then place the thumb over the mouth of the tube and invert it several times to insure a perfect mingling of the urine with the reagent. The tube should now be placed in a special receptacle (Fig. 87) and allowed to stand for twenty-four hours, when the sediment which collects at the bottom of the tube will consist of serum-albumin, serum-globulin, albumoses, uric acid, and kreatinin. The amount of sediment is read directly from the scale, and indicates the amount *pro mille* in grams.

Wetherill has designed the torfuge (p. 528, Fig. 233) so as to contain Esbach's albuminometer, and by the use of this instrument the reading may be obtained in from one to two minutes.

Cautions.—Where the specific gravity of the urine is above 1.008 the specimen should be diluted with water to reach this density. The temperature of the room should be 15° C. (59° F.). The urine must be acid.

Different Densities.—The urinary albumin may be estimated by obtaining the specific gravity of the urine as voided, and after it has been freed from albumin.

Application.—1. Treat a quantity of urine of known specific gravity with sufficient acetic acid to precipitate the albumin, and note the specific gravity.

2. Place the solution in a rubber-stoppered bottle (the stopper having been previously boiled in sodium hydrate and washed to remove all alkali), tightly fasten it, and hold it in position by a wire.

3. Place the bottle in boiling water for from ten to fifteen minutes.

4. Cool and filter, guarding, as much as possible, against evaporation by passing the urine through a funnel surrounded by a closely fitting stopper, and by keeping the funnel covered with a glass plate.

5. Ascertain the specific gravity of the filtered urine—it is best to employ a pyknometer. Multiply the decrease in specific gravity by 400 and the product equals the number of grams of albumin present in 100 c.c. of urine.

Gravimetric Method.—1. Take 500 to 1000 c.c. of acid filtered urine (if alkaline, acidify with acetic acid) and place a few cubic centimeters of this quantity in a test-tube, heat in boiling water until coagulation occurs, heat again over a flame, and filter.

2. Test the filtrate with acetic acid and potassium ferrocyanid. Should no precipitate form, treat the entire quantity of urine in the same manner, no further addition of acid being required.

3. In the event of albumin being demonstrable with acetic acid and potassium ferrocyanid, the entire volume should be treated with a 30 per cent. solution of acetic acid (adding a few drops at a time) and stirring with a glass rod after each addition.

4. After having proved that the urine is sufficiently acid to allow a complete precipitation of the albumin present, place 100 c.c. or more in a rubber-stoppered bottle (see (2) *Method of Different Densities*), and first heat in boiling water until the albumin is seen to separate in flake-like particles. Then remove the bottle from the water, wipe dry, and again boil.

5. Decant the supernatant urine, passing it through a dried, weighed filter, and later bring the entire precipitate upon the filter, care being taken to detach all particles of albumin that may cling to the beaker by means of a glass rod tipped with rubber tubing and by washing with hot water.

6. Wash the precipitate until the washings are no longer turbid, and treat with a drop of nitric acid and silver nitrate. Again wash with alcohol, and with ether remove all fats, after which the filter is dried at 120° to 130° C. (248° to 266° F.) and weighed. Should greater accuracy be required, incinerate the dried and weighed precipitate and thus determine the amount of mineral ash present with the albumin, and deduct this amount from the original weight.

Whenever possible it is well either to dilute the urine sufficiently that not more than 0.2 to 0.3 gm. of albumin be contained in the quantity of urine employed, or to employ less than 100 c.c. of urine, thereby accomplishing this end.

Serum-globulin.—Test.—1. Place from 20 to 50 c.c. of filtered urine in a beaker, and render it alkaline by adding ammonium hydrate. Phosphates are thus thrown out of the solution and should be separated by filtering.

2. Add to the urine an equal quantity of a saturated solution of ammonium sulphate. A precipitate is indicative of the presence of globulin. Ammonium urate may also be separated from the solution, but is placarded by its color (see *Ammonium Urate*, page 272).

Paton's Test.—After the urine has been rendered alkaline and the phosphates separated as previously outlined, place 10 c.c. of a saturated solution of sodium sulphate in a test-tube and allow some of the urine to flow in along the side of the tube so as to form a layer above the reagent. Serum-globulin causes a white ring to appear at the zone of contact.

Quantitative Estimation.—After serum-globulin has been precipitated by one of the methods described, allow the mixture to stand for one or more hours, collect the precipitate on a dried and weighed filter, and wash with a half-saturated solution of ammonium sulphate until the washing fails to give a precipitate, when treat with acetic acid and potassium ferrocyanid. Continue as in the quantitative estimation of serum-albumin, page 211.

Mucin (Nucleo-albumin).—Nucleo-albumin differs from globulin in that it is insoluble in acetic acid. It is precipitated by magnesium sulphate, and does not form reducing substances when boiled with dilute acids, which also serve to distinguish it from true mucin.

Place 10 c.c. of filtered urine in a test-tube and add concentrated acetic acid in excess—turbidity indicates the presence of nucleo-albumin. Should serum-albumin be present, it is to be removed by boiling and filtering, after which dilute the urine 1 to 3 of water, which prevents the possibility of turbidity resulting from urates, and lessens the power of the urinary salts to dissolve the mucin.

Ott's Test.—*Reagent.*—Almén's solution:

℞. Tannic acid	5 gm.
Sol. acetic acid (25 per cent.)	10 c.c.
Alcohol (40 to 50 per cent.)	240 c.c.

M. and keep in a glass-stoppered bottle.

Place 10 c.c. of the filtered urine in a beaker and add an equal volume of a saturated solution of common salt. Shake well and then slowly add a small quantity of Almén's solution, when, should nucleo-albumin be present, a precipitate appears immediately.

The following table may serve to differentiate between mucin, nucleo-albumin, and pus, though the two former of these bodies appear to be of questionable nature and have therefore been considered under one head.

MUCIN.	NUCLEO-ALBUMIN.	PUS.
1. In acid urine mucin forms a loose, whitish precipitate.	1. Spontaneous precipitate rare. Caused by magnesium sulphate.	1. Thick white or yellowish-white precipitate in acid urine.
2. When urine is rendered alkaline, precipitate is partially or entirely dissolved.	2. Not influenced.	2. Unaltered.
3. Caustic potash causes no further change.	3. Slightly influenced.	3. Causes the precipitate to become stringy or ropy.
4. Microscopic examination negative.	4. Negative.	4. Pus-cells showing various stages of degeneration.
5. Insoluble in excess of acetic acid.	5. Feebly soluble in large excess.	5. Insoluble.
6. A diffuse or indistinct hazy band at zone of contact with nitric acid applied by Heller's and the author's methods.	6. A sharp ring at zone of contact.	6. An albumin ring the rule.
7. Yields a reducing substance when boiled with mineral acids; and its ash does not contain phosphorus.	7. Does not yield reducing substances as a rule. Its ash contains phosphorus.	7. Unimportant. Does not contain phosphorus.

Clinical Significance.—Nucleo-albumin, or a substance closely allied to it, and mucin are normal constituents of the urine, and are to be found in large amounts during the course of catarrhal processes of the urinary tract, especially of the bladder.

Emulsion-albuminuria.—Under this caption Cramer* has described a unique condition of the urine. In this condition the urine is chylous in appearance, but when placed under the microscope its turbidity is found to be due to a peculiar hazing of the entire field caused by innumerable minute globules of fat. When extracted with ether no fat is to be obtained and the osmic-acid reaction is negative. After the evaporation of the ethereal solution an almost colorless residue results, which in one instance—Coriat's case †—emitted an odor of burned sugar. Fehling's solution gives a doubtful reaction, and Nylander's (bismuth) test is negative. There is no formation of an osazone with phenylhydrazin and sodium acetate (page 229). No volatile liquid is obtained by destructive distillation (*acrolein test*). The milkiness of such urine is changed to a mere opalescence upon the addition of a few drops of acetic acid or of potassium hydrate. Dilution of the urine with several times its own volume of water also causes a mere opalescence.

Urine acidulated with hydrochloric acid and heated upon a water-bath for one hour may deposit a crystalline substance which is probably allantoin. This body appears to be more closely allied to crystalline globulin; the crystals occasionally found in urines containing Bence-Jones' albumose; and also the colloidal solution of metals, where, by electric pulverization of such metals as platinum, silver, and iridium, they become mechanically suspended as fine particles in water or other fluids. The urine partly cleared by dilution or otherwise gives the ordinary reactions for proteids.

Upon boiling, a copious precipitate is obtained; the supernatant liquid first becoming opalescent and eventually clear. Artificial peptic digestion also clears the urine; but it remains milky upon the addition of hydrochloric acid alone.

Microscopy.—The sediment of such urine is likely to contain crystals of uric acid, amorphous urates (few), leukocytes, and in one instance pus and a few erythrocytes were found.

Thus far I have been able to recover from the literature reports of but four examples of emulsion-albuminuria.

Albumoses.—Acidify 10 to 20 c.c. of urine with acetic acid and to it add a saturated solution of sodium chlorid, and shake.

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* "Münchener med. Wochensch.," Jan. 21, 1902.

† "Med. Rec.," Nov. 14, 1903.

Albumoses cause a precipitate to appear upon gentle application of heat before the boiling-point is reached, but should ebullition be prolonged, this precipitate is dissolved in part or in its entirety, to reappear on cooling; and when filtered hot, the filtrate becomes cloudy on cooling. Render the hot filtrate alkaline with sodium hydrate and to it add, drop by drop, a 1 per cent. solution of copper sulphate; continue to heat, and a red color develops (biuret reaction).

Millon's Reaction.—*Millon's Reagent.*—

Mercury	1 part
Nitric acid (specific gravity 1.42)	2 parts
Distilled water	6 "

Place 10 c.c. of urine in a test-tube and to it add a small quantity of Millon's reagent; boil briskly and a red color develops. (Allusion to further tests for albumoses is made under *Serum-albumin*, page 210, and in connection with Bence-Jones' albumose, page 218.)

Peptones.—Peptones, in the opinion of Kühne, do not occur in the urine, but the substance generally regarded as peptone is classified under this caption for want of a more appropriate term.

Test.—*Salkowski's Method.*—1. Place 50 c.c. of the urine in a beaker and acidify with 5 c.c. of hydrochloric acid; add phosphotungstic acid, heating over a flame for five minutes, when a precipitate will collect in the form of a resinous mass adhering closely to the bottom of the beaker.

2. Decant supernatant fluid, wash the precipitated mass twice with distilled water, decanting after each washing.

3. Add to the precipitate 8 c.c. of distilled water and 0.5 c.c. of sodium-hydrate solution (specific gravity 1.16). Upon shaking the beaker and stirring the mass with a glass rod it is readily dissolved, and the solution assumes a dark-blue color.

4. Heat over the flame for several minutes, when the blue color is replaced by a dirty grayish yellow, and the solution becomes turbid, though rarely it remains clear. At this point place a few cubic centimeters of the solution in a test-tube, cool, and treat with a 1 per cent. solution of copper sulphate (drop by drop) when a bright-red color (*biuret reaction*) appears. Albumin and mucin should be removed from the urine (see page 206), though the latter substance seldom interferes with the reaction. By this method 0.015 gm. of peptone per 100 c.c. may be readily demonstrated.

The biuret reaction is likewise obtained with the urine of pneumonia and other febrile conditions. When question arises, not more than 10 c.c. of urine should be employed for the reaction. Hematoporphyrin (page 223) will be found to interfere with this reaction, in which case the urine must be precipitated by barium chlorid, an alcoholic extract obtained, and the filtrate, which holds in suspension the albumoses, examined.

Clinical Significance.—Peptone may appear in the urine as a result of pathologic conditions, and its presence is probably dependent upon the decomposition of organized proteids, except in cases of the enterogenic form (constipation). It may owe its origin pathologically to the action of bacteria upon the tissues, or to chemic poisons (phosphorus), and it may be encountered with the various cirrhoses of the liver, acute yellow atrophy, gastric cancer, gangrene, acute or chronic suppuration, acute inflammation of serous surfaces, leukocythemia, infectious fevers, after death of fetus, and physiologically during involution of the uterus.

Bence-Jones' Albumose.—This form of albumosuria, though extremely rare, is doubtless worthy of special consideration, and it is the author's belief that a more definite knowledge of the reactions common to this body will facilitate its recognition, thereby elucidating a number of obscure conditions. Since the original report of Henry Bence-Jones, there have appeared in the literature, from time to time, reports of thirty-five cases of Bence-Jones' albumosuria; four of these having been studied by the author.*

Tests.—1. Place from 10 to 20 c.c. of filtered urine in a test-tube and heat, allowing the flame to warm the upper stratum of the urine. Bence-Jones' albumose causes an opalescence to appear long before the boiling-point is reached, and when near the boiling-point, the fluid becomes opaque, and coarse flocculi are seen falling to the stratum of unheated urine. When the boiling-point is reached this turbidity will be seen to diminish slightly. When ebullition is prolonged for from one to three minutes, a portion and, sometimes, nearly all the turbidity disappears; but upon standing the tube aside and allowing the urine to cool, this clouding reappears and is often more pronounced than before the boiling-point was reached.

2. Place from 10 to 40 c.c. of the filtered urine in a beaker and heat over a water-bath, keeping careful watch at what temperature the opalescence is observed. Bence-Jones' albumose causes a slight cloud at 52° C. (125.6° F.), and at 54° to 56° C. (129.2° to 132.8° F.) a marked turbidity appears, which develops into a dense cloud at from 56° to 60° C. (132.8° to 140° F.). A rather

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* Phila. Col. Physicians, June 4, 1902; Anders and Boston, "Lancet," Jan. 10, 1903; "Amer. Jour. Med. Sci.," April, 1903.

tough coagulum formed in one urine examined when heated from 60° to 90° C. (140° to 194° F.). According to some writers, the addition of a few drops of acetic acid to the urine may cause the opalescence to appear at a lower temperature (37° C. or 98.6° F.), though this has not been my experience. The coagulum or precipitate, however dense, will be found to dissolve in part when kept from two to six minutes at a temperature of 95° to 100° C. boiling.

3. Upon the addition of concentrated nitric acid, drop by drop, to the urine, the upper one-third of which has been gently heated, a precipitate appears in the track of the acid as it travels through the cold stratum of the urine to the bottom, where after the addition of several drops, a rather dense precipitate of a slight pink or yellowish hue collects. In some specimens this precipitate upon standing becomes a golden color.

4. Add to the urine nitric acid, drop by drop, shaking after each additional drop, when a slight pinkish tint appears affecting mostly the heated portion of the urine.

5. Place 20 c.c. of urine in a test-tube, acidulate with acetic acid, and heat gradually to the boiling-point, cool, and filter. The filtrate thus obtained apparently contains but little proteid, displaying a slight opalescence with acetic acid, but no precipitate appearing with nitric acid. Nitric acid when added to such filtrate may lend a pinkish tint to the fluid, which upon the addition of ammonia often assumes a yellowish shade.

6. Place 50 c.c. of the urine in a beaker, heat over a water-bath to 54° C. (129.2° F.), and filter. Dissolve the precipitate that collects on the filter by washing with a solution of caustic soda (30 per cent.); and then test for Millon's and the biuret reactions (Millon and biuret tests, page 217).

7. This precipitate when heated with lead acetate (1 per cent. solution) and caustic soda (30 per cent.) first displays slight browning, but after the boiling has been prolonged for one or more minutes, the color gradually deepens to a dull black due to the formation of lead sulphid. Matthes, Milroy, Magnus Levy, and Huppert regard this reaction as dependent upon the fact that the precipitate contains sulphur (loosely combined) in considerable amount and as one of the most characteristic features of Bence-Jones' albumose.

Saturation with Neutral Salts.—1. Place a quantity of filtered urine in a beaker and to it add an equal volume of neutral solution of ammonium sulphate which will be found completely to precipitate the albumose.

2. Saturation with sodium chlorid (equal volume) in neutral

solution causes a precipitate of but a portion of the albumose; the filtrate clouding decidedly upon the addition of a small quantity of dilute acetic acid; but the filtrate from this second precipitate does not contain albumose. Bence-Jones' albumose differs from proto-albumose and hetero-albumose in that it is not completely precipitated with sodium chlorid in neutral solution. It resembles hetero-albumose in that it is precipitated on the addition of acetic acid after saturation with sodium chlorid or magnesium sulphate, but differs from this body in being precipitated at a temperature of 52° C. (125.6° F.).

Bence-Jones' albumose is precipitated by sulphuric, hydrochloric, tannic, and picric acids. Reactions 5 and 6 will be found equally decisive after the urine has been saturated with neutral salt. Place 50 c.c. of the filtrate thus obtained, which contains albumose, in a beaker and add a drop of hydrochloric acid; stir gently, after which a few drops of phosphotungstic acid should be added and heat gradually applied, which when prolonged for a few minutes causes a copious precipitate to collect at the bottom in the form of a rather tough, whitish mass. Decant the clear supernatant fluid, and dissolve this precipitate in a 30 per cent. solution of caustic soda, adding drop by drop. This usually gives a colorless mixture, but occasionally it will be found to display a blue or purple tint, which disappears when the specimen is gently heated.

Special Tests.—The Millon, biuret (page 217), and sulphur reactions may now be continued as described under 5 and 6. Kühne and Matthes found Bence-Jones' albumose to contain phophorus in rather large amounts, while Milroy was unable to recover phosphorus in his studies. Simon has subjected this body to peptic digestion.

Histon.—Bence-Jones' albumose resembles in many respects histon, which under certain conditions may be derived from nucleo-histon, a proteid of the leukocytes. Milroy regards histon to be an intermediate between albumin and albumose. Histon is precipitated from acid solutions by ammonia, while albumose causes no precipitate. Histon when heated yields a precipitate which is soluble in dilute acids. It is precipitated on the addition of nitric acid; this precipitate disappearing when heated to the boiling-point, to reappear on cooling. It gives a decided rose-pink color with copper sulphate and caustic soda (biuret).

Globin.—Shultz and Milroy have studied correlatively the reactions for Bence-Jones' albumose and for globin, and found these bodies to behave alike when treated with strong hydrochloric and nitric acids, and also to give the biuret reaction. Albumose gives a distinct red with Millon's reagent, globin a pink; and albumose contains loosely combined sulphur. Globin when precipitated by heat is readily soluble in dilute acids, a feature less prominent in albumose.

Clinical Significance and Peculiarities.—Bence-Jones' albumosuria is a condition which manifests itself after the age of twenty years. Males are affected in 80 per cent. of the cases. In 15 per cent. of cases there is a history of traumatism inflicting severe injury to the bony skeleton. Primary lesions of the bones (myeloma) have been found postmortem in 80 per cent. of cases.

This condition (albumosuria) may be persistent, transitory, or, less commonly, remittent, occurring in variable degrees at different hours during the day. The specific gravity of the urine may fluctuate between 1.002 and 1.040, and there appear records of six instances where the urine resembled syrup. The color of the urine varies greatly. The quantity for the twenty-four hours is usually normal, though polyuria may develop.

Maladies displaying albumosuria are usually fatal in from one to two and one-half years.

Hematuria.—Hematuria is a condition wherein the cellular elements of the blood escape through the urine, giving to this secretion a bloody color which upon standing becomes of a more or less blackish or brownish red, and at the bottom of the liquid there is seen a chocolate precipitate containing blood-cells and often blood crystals. The reaction of the urine modifies the microscopic character of the precipitate.

Heller's Test.—This test depends upon the presence of blood-pigment and therefore gives a positive reaction with both hematuric and hemoglobinuric urines. Place a test-tube in urine to the height of two inches and render strongly alkaline with caustic soda (30 per cent.), after which boil. Blood-pigment gives a brownish-red deposit, while the supernatant fluid becomes bottle-green. This precipitate consists of earthy phosphates and hematin derived from the blood-pigment present, and is therefore reddish in color instead of being white or yellow as would be the case were only phosphates deposited. Alkaline urine should first be treated with a few drops of calcium-chlorid solution. When the color of the urine is very dark, due to the presence of pigments of blood or bile, decant the supernatant fluid and add to the precipitate an equal volume of water. This test is capable of detecting 1 c.c. of blood in 1 liter of urine.

Fallacies.—When the patient is taking senna, santonin, or rhubarb, a positive reaction is obtained with the urine in the absence of blood-pigments and it is necessary to resort to spectroscopic analysis to distinguish between these reactions and that of blood.

Hemoglobinuria.—Hemoglobinuria is a condition marked by the presence of hemoglobin or its derivative, methemoglobin, in the urine without the presence of red blood-cells, and is excited by the conditions capable of inducing hemoglobinemia (page 141), among which malaria deserves special mention. It may occur where the urine shows a small number of red cells microscopically, the urine containing hemoglobin whenever the red cells are present in sufficient numbers. The specific gravity of such urines is commonly low (1.008), but in exceptional instances it may reach 1.030. Bloody urine is turbid and therefore placarded by its color, which may vary from a bright red to almost a black. Oxyhemoglobin can be recognized as such by the spectroscope, since it gives rise to the appearance of two absorption-bands located between D and E (Fig. 24).

Microscopically, red blood-cells are rarely present. The field contains much granular debris, among which are seen many small droplets which are often of a yellowish color. Coarse clusters of yellowish matter are to be seen, as are also crystals of hematoidin (Plate 17) and epithelial cells containing granules of pigment.

Spectrum.—Render a quantity of the suspected urine acid by adding a few drops of acetic acid, place in a test-tube, and hold before the open slit of the spectroscope, when the bands of oxyhemoglobin will be seen. Should these bands not appear at once, dilute the urine with distilled water until they become perceptible. Add a few drops of ammonium sulphid to the urine and the spectrum is converted to that of reduced hemoglobin. In my own experience it has been common to obtain a spectrum of reduced hemoglobin (Fig. 24) in cases of hemoglobinuria.

Guaiacum Test.—Place one inch of urine in a test-tube and allow a mixture of equal parts of tincture of guaiacum and oil of turpentine that has been ozonized by exposure to the air to flow from the pipet along the side of the test-tube so as to form a distinct layer upon the urine. Blood-pigment causes a white ring to appear at the zone of contact, which soon changes to a blue, due to oxidation of the guaiac by oxygen derived from the ozonic ether, the blood-pigment acting as a carrier. (Ozonic ether is a solution of peroxid of hydrogen in sulphuric ether.)

Fallacies.—Should the urine contain iodids, the blue color also appears with this test and is to be distinguished from the reaction due to blood by the fact that the color appears much more slowly and in addition develops simultaneously throughout the fluid instead of at the junction of the urine and the ether. In the presence of pus a greenish-blue color appears, but the urine is decolorized on heating. Where the urine has been voided in a vessel into which sputum has been collected, saliva will be found to cause this reaction with guaiacum.

Methemoglobinuria.—Methemoglobin is a derivative of the blood which may be formed from hemoglobin, in acid urine, after it has been allowed to stand for several hours; and it is not uncommon to find methemoglobin in the freshly voided urine. Clinically it has been regarded as indicative of renal hemorrhage. The urine has a smoky tint, and upon spectroscopic study (see *Blood*, page 45) furnishes the only satisfactory test for methemoglobin. For this purpose it is well to filter, and also to dilute, the urine.

Hematoporphyrinuria.—Iron-free pigment (hematin) occurs normally in the urine, though in but minute amounts, and may be considerably increased without noticeably altering the color of the urine, while in hematoporphyrinuria the urine has a port-wine color and does not give the guaiac reaction. Spectroscopically, when studied in a thin layer, it may show a characteristic spectrum of the so-called "alkaline hematoporphyrin" (Fig. 24), and in this way may be encountered in acid urines. It is not uncommon, however, for spectroscopic study to yield negative results, and in such instances the pigment can be extracted by shaking the urine with a small amount of amylic alcohol or acetic ether, after the addition of one or two drops of acetic acid. The extract thus obtained when studied spectroscopically reveals the bands of alkaline hematoporphyrin, which are four in number: one at the junction of the red and yellow between C and D, a second in the yellow, a third in the green both between D and E, and a fourth, darkest and broadest, appearing between E and F, and the green and the blue (see Fig. 24). The bands of hematoporphyrin may be obtained by the addition of one or more drops of hydrochloric acid, and are two in number: one narrow band in the orange between C and D, and another broader band at the junction of the yellow and the green between D and E, which is the characteristic band. In reality this consists of two halves, a lighter, situated nearest the narrow band and a very dark, located half way from it.

Clinical Significance.—Hematoporphyrin may appear in large amounts in the urine of persons who have been taking sulphonal.* It is said to be more common in females than in males and should be regarded as a grave symptom.

Histon.—Collect the product of twenty-four hours and examine first for serum-albumin and albumose, as has been previously outlined (page 200). Precipitate the histon with from 90 to 94 per cent. alcohol, and wash the precipitate with hot alcohol and dissolve in boiling water; cool, acidify with a few drops of hydrochloric acid, and set aside for several hours. During this time clouding and decided precipitate appear, composed largely of uric acid (which has to be separated by filtration), and the filtrate, precipitated with ammonia, which throws out of solution histon and certain other constituents of the urine. Collect the precipitate on a filter, and wash with ammoniacal water until the wash-water fails to give the biuret reaction. Dissolve the precipitate in dilute acetic acid and test the solution for the biuret reaction. Should this be positive and coagulation occur upon the application of heat, and the coagulum be soluble in mineral acids, histon is probably present (see *Differential Points for Histon and Bence-Jones' Albumose*, page 220).

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* Tyson and Croftan, "Phila. Med. Jour.," May 17, 1902, p. 882.

Fibrin.—Fibrin may occur in the urine in the form of rather decided clots, and their character is quite distinct after thorough washing with water. Clots of fibrin are dissolved by boiling in a 1 per cent. solution of soda or in a 5 per cent. solution of hydrochloric acid. After the dissolution of these fibrinous coagula, test the urine for serum-albumin (page 206).

CARBOHYDRATE (SUGAR) IN THE URINE.

The sugars that occur in the urine which are of practical importance from a clinical point of view are glucose and lactose. It is possible, however, that levulose sometimes occurs in conjunction with glucose, and cane-sugar and maltose may appear in the urine after the ingestion of a large amount of either of these substances. Their occurrence is commonly described under the special caption of pentoses (page 238). Their clinical importance is at present undetermined.

GLUCOSE.

Glucose (dextrose or grape-sugar, $C_{16}H_{12}O_6$) forms by far the commonest variety of sugar encountered in the urine, and its presence there is commonly referred to as "glucosuria," yet properly speaking glucosuria is not true diabetes mellitus unless glucosuria forms the most conspicuous feature of the condition. A mere trace of glucose may be met with in normal urine, but not in sufficient amounts to render it capable of detection by the reagents commonly employed for this purpose. Therefore, whenever a reaction for glucose is obtained by our present laboratory methods, a pathologic glucosuria exists.

Clinical Significance.—Glucosuria may be persistent or transitory, the latter form of which will be divided into three classes for the convenience of study.

Transitory Glucosuria.—It is quite impossible within the scope of this volume to discuss in detail the various forms of transitory glucosuria, and therefore mere allusions to such conditions will be made. As previously stated, there is a faint trace of glucose in normal urine, and it has been further found that after breakfast, if the meal be rich in carbohydrates, sugar is usually demonstrable in the urine. In addition to diabetes mellitus, the causal factors of which are not herein discussed, there are to be encountered the so-called alimentary glucosurias, and in certain low-grade conditions transitory glucosuria is not an unusual occurrence. For convenience of study these transitory glucosurias may be classified under the following subheadings: (*a*) toxic; (*b*) those associated with disease or injuries to the human organism; and (*c*) puerperal glucosuria.

Toxic glucosuria is to be seen after the administration of hydrochloric acid, sulphuric acid, mercury, strychnin, glycerin, alcohol, nitrobenzol, lead, arsenic, phosphorus, potassium iodid, caffein, thyroid extract, tuberculin, pancreatin, phloridzin, diuretin, carbon monoxid, and morphin. Analgesics and anesthetics also possess the power of exciting transitory glucosuria, and several instances are recorded where glucosuria has followed the administration of chloral, chloroform, and amyl nitrite; and three observers have found small quantities of sugar in the urine after ether anesthesia, while Andral reports a case of true diabetes developing after ether anesthesia.

When the urine is capable of reducing copper after the administration of any of the above-named drugs, the phenylhydrazin test should be employed, as there are likely to be other reducing agents present in such urines.

Among the pathologic conditions wherein transitory glucosuria has been detected should be mentioned cerebrospinal meningitis, in which disease I have found it to be of a rather common occurrence; cholera, relapsing fever, typhoid fever, diphtheria (in which I have seen two instances), phthisis, certain of the exanthemata, as scarlet fever, hepatic cirrhosis, rickets, gastritis, during malarial paroxysms, scarlatinal nephritis, chronic interstitial nephritis, cholelithiasis, syphilis, asthma, whooping-cough, and, according to the late Da Costa, in old age. Again, glucosuria may follow injuries to the cerebrospinal system and cerebral or pontine hemorrhage, traumatism to the head, cerebral abscess or tumor. It may occur in disseminated sclerosis, epilepsy, neuralgias, sciatica, and the various forms of insanity. It has also been observed during the course of exophthalmic goiter, myxedema, and neurasthenia; as well as following traumatic neuroses, physical derangements, and anxiety or sudden emotions. The term functional glucosuria has been applied where glucosuria develops in connection with many of these conditions, but in all instances it is liable to be transitory in nature.

Puerperal lactosuria is much more common than is glucosuria, although the latter condition is but rarely seen (see *Lactosuria*, page 238). This form may possibly be toxic, although it is physiologic unless the quantity of sugar be large. Roque* suggests the following classification of glucosuria:

Intermittent glucosuria of arthritis, which should probably be included with hereditary glucosuria of the young, as well as that of gouty adults and obesity.

Digestive glucosuria is simply recognized by its disappearance after the withdrawal of carbohydrates from the diet and the removing of digestive disturbances.

Nervous glucosuria, including those conditions associated with systemic affections involving the nervous system, neuroses, psychoses, and traumatism.

Tests for Glucosuria.—Many of the tests for glucose depend upon the fact that these substances become oxidized by the expenditure of certain metallic oxids; such oxidation occurring easily at a temperature near the boiling-point and in the presence of a free caustic alkali. Copper serves as the most suitable metal for this test. Take a solution of caustic soda and to it add a few drops of a very dilute solution of sulphate of copper—a blue precipitate develops which is hydrated cupric oxid ($CuOH_2O$). Upon boiling, this blue precipitate changes to black from the separation of cupric oxid (CuO). When in the presence of certain substances, such as tartrate, the cupric hydrate previously formed on the addition of the sulphate is retained in solution instead of being precipitated, and gives the fluid a deep blue color which is unaltered by boiling. In the presence of oxidizable substances, as glucose, the blue cupric hydrate is reduced, when the temperature is raised to near the boiling-point, to form cuprous hydrate (Cu_2OH_2O), which when thrown out of the solution appears as a yellow precipitate; or should it be further dehydrated, cuprous oxid (CuO) forms, which is red.

Caution.—The solution containing glucose when rendered alkaline with caustic soda and treated with a few drops of cupric-sulphate solution causes cupric hydrate to be formed, which may be dissolved and the blue solution remain unchanged, but when heated to the boiling-point glucose reduces the cupric hydrate and the yellow cuprous hydrate or the red cuprous oxid appears as a precipitate.

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* Paris, 1899.

Fehling's Test.—*Reagents.*—Solution I: Powder 34.64 gm. of pure crystallized sulphate of copper, and dissolve in 200 c.c. of warm distilled water; cool, and add distilled water to make 500 c.c.

Solution II:

Crystallized Rochelle salts	180 gm.
Distilled water (hot)	300 c.c.

filter, and add 70 gm. of pure caustic soda ; cool, adding sufficient water to make 500 c.c. The solutions should be kept in colored glass-stoppered bottles.

Preparation of Urine.—I. Place 10 c.c. of urine in a beaker, boil, and without removing any precipitate add to it 5 c.c. of the copper-sulphate solution used in preparing Fehling's solution, and after cooling add 0.5 c.c. of a saturated solution of sodium acetate which contains sufficient acetic acid to give to the mixture a rather feeble acid reaction. Filter. Employ the filtrate in testing with Fehling's solution as outlined below. The urine must be freed from albumin, and it is well to add a few drops of acetic acid; boil and filter, after which neutralize the filtrate with calcium carbonate.

II. Fehling's test is not applicable to ammoniacal urine, as the free ammonium may prevent the precipitation of cuprous oxid. When the glucose present is more than necessary for the reduction of all the cupric oxid, the remaining glucose, should ebullition be prolonged, is liable to be caramelized, in which case both the liquid and the precipitate assume a dark brownish color. Mix equal volumes of solution (I) and (II) which results in an *alkaline solution of potassic cupric tartrate*, 1 c.c. of which is reduced by 5 mgm. of pure glucose. When preparing for qualitative analysis add to this mixture three times its volume of water, which will result in a pale-blue solution.

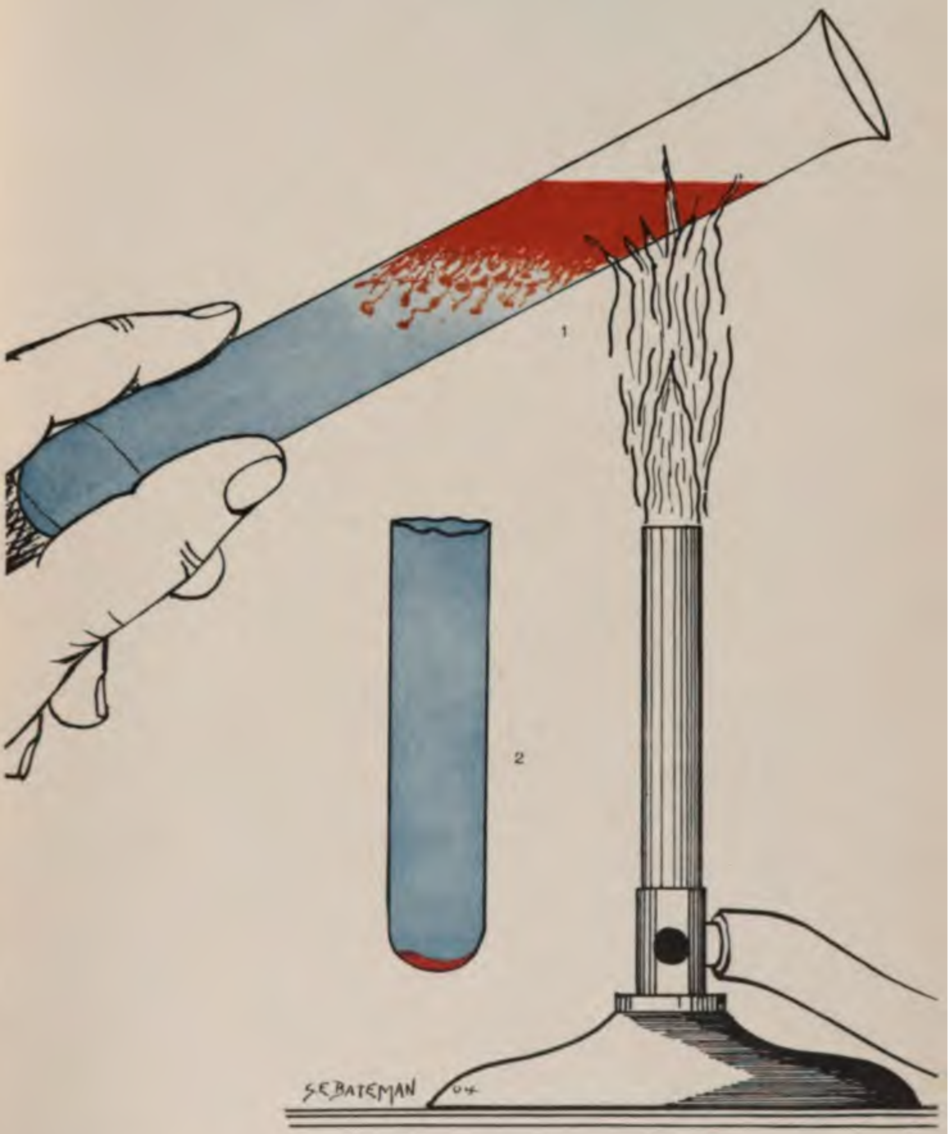
III. Fill a test-tube two-thirds full with the diluted Fehling's reagent, and boil the upper portion of the reagent for one minute. If the reagent remains clear it is fit for use, but should the slightest turbidity ensue, its employment is unsafe, and caustic soda should be added to the solution, after which filtration is necessary before use.

IV. Grasp the tube containing the reagent between the thumb and finger (see Plate 14), and heat the upper portion of the liquid over a free flame, at the same time adding, drop by drop, urine from a pipet, and heating after each additional drop of the suspected *albumin-free* urine. In the presence of a considerable quantity of glucose, a yellow or red precipitate suddenly develops, its color changing gradually according to the quantity of glucose present, as well as to the dilution of the copper solution and the presence of other substances, such as uric acid and kreatinin. From 5 to 20 drops are sufficient to cause a reduction of the copper when glucose is present in pathologic amounts, yet more urine is often required. As the reduction develops, small drops of the urine will be seen falling from the under surface of the reaction (precipitate) through the clear reagent and will cause a precipitate to form in the track of the urine as it traverses the reagent toward the bottom of the tube (Plate 14). This occurs only when glucose is present in considerable amounts, but when seen is a most characteristic feature. Such reactions after standing for a time are likely to show a diminishing of the yellow precipitate suspended near the top of the tube, while at the bottom a rather coarsely granular sediment collects (Plate 14).

Cautions.—Never boil the upper portion of the urine briskly and do not heat the lower portion, since boiling obscures the characteristic features of the reaction, and renders the significance of the copper reaction questionable. Should no decoloration of the copper solution follow the addition of the urine, continue until the quantity of the urine that has been added nearly equals in volume that of the reagent. Boil gently for a few seconds and set aside. If upon cooling no precipitate develops, sugar is absent. When the precipitate develops late, the liquid first shows slight clouding and passes through the various stages from a clear bluish green to a light-green color, which often appears milky and is by some authors regarded as rather characteristic of dextrose, though it is more likely the result of some additional substance which interferes with the characteristic reaction. Fehling's test is capable of detecting 0.8 per cent. of glucose, in which case the precipitate is yellow or red, but the color does not form until the liquid cools.

Other Substances.—Copper is capable of being reduced by uric acid, kreatinin, calcium oxalate, and hippuric acid, all of which are constituents of normal urine (and among abnormal ingredients are to be mentioned lactose, glycuronic acid, glycosuric acid), and following the administration of such drugs as chloroform, chloral, benzoic acid, salicylic acid, potassium iodid, salicylates, glycerin, and carbolic acid (see *Alkaptonuria*, page 247). When the precautions previously set forth have been observed, there are but two substances likely to confuse the reac-

PLATE 14.



FEHLING'S TEST.

1. Method of applying heat when using Fehling's reagent; note the lower surface of the reaction. 2. Showing the result of standing.

tions for glucose, viz., glycuronic acid and lactose, a point to be determined by the following tests:

Nylander's or Böttger's Test.—This reaction depends upon the power of glucose to reduce the bismuth compounds when in alkaline solution, with the formation of the black suboxid.

Reagent.—Dissolve 10 gm. of caustic soda in 90 c.c. of warm water, and add 4 gm. of sodiopotassium tartrate and 2 gm. of bismuth subnitrate. After shaking thoroughly a hydrated oxid of bismuth is formed and is held in solution by the tartrate. Boil, filter, and keep in a glass-stoppered amber bottle. Place 10 c.c. of urine in a test-tube and to it add 1 c.c. of the reagent, shaking sufficiently to cause a thorough mingling of the reagent through the urine; then boil over a flame for from one to three minutes. Sugar causes at first a brown and later a black precipitate of bismuthous oxid (metallic bismuth) to be thrown down. This reaction, while extremely delicate, is less liable to occur with the substances normally found in the urine, and mentioned under Fehling's test. It does occur as the result of glycuronic acid, lactose, after the administration of rhubarb, salol, antipyrin, senna, and turpentine; and when urine contains an abundance of oxalate of calcium, a dark-brown precipitate is produced. Urines containing traces of sulphur also give this reaction, and albumins favor the precipitation of the black sulphid of bismuth.

Picric-acid Test.—Place 10 c.c. of urine in a test-tube and to it add one-fourth its volume of a standard solution of picric acid; shake, and heat after adding a few drops of caustic potash. In the presence of sugar the solution assumes a dark-red color which is dependent upon the conversion of picric to picramic acid.

Caution.—The kreatinin contained in normal urine may cause some darkening of the fluid, but the color does not resemble that produced by sugar, and the solution, while colored, does not become cloudy. Any impurity of the reagent may show spontaneous darkening when heated with caustic potash.

Phenylhydrazin.—Place in a beaker one-half gm. of phenylhydrazin hydrochlorate (colorless crystals) and 1½ gm. of sodium acetate with sufficient water to dissolve, with the aid of gentle heat over a water-bath; then to this solution add 5 c.c. of the suspected urine and boil the mixture for from three to five minutes; after which set aside and cool. Should sugar be present the crystals will soon accumulate at the bottom of the liquid. A step which I prefer is to centrifugate some of this urine in order to recover the crystals for microscopic study. These crystals of phenylglucosazone appear as small needles, are of a bright yellow color, and may appear singly, though commonly tend to congregate in rather dense aggregations, often arranging themselves in bundles, sheaves, stars, and fans. They are seen with a one-sixth lens. The phenylhydrazin test is sufficiently delicate for the demonstration of 0.01 per cent. of glucose. The melting-point of phenylglucosazone is 205° C. (401° F.), while maltosazone melts at 190° to 191° C. (374° to 375.8° F.).

Caution.—Maltose and certain pentoses are also capable of causing the formation of osazone. Crystals of lactose are, as a rule, short, heavy, and pointed at both extremities, and unless the sediment be centrifugated, show little tendency to form aggregations.

Rigler's Test.—Place one and one-half gm. of pure phenylhydrazin crystals and 7 gm. of sodium acetate in a porcelain capsule, and boil after adding 20 drops of the suspected urine. Remove the capsule to the table and add 20 to 30 drops of a solution of caustic soda (10 per cent.). In the presence of sugar a red-violet color appears within one minute.

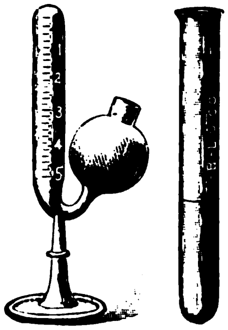


Fig. 89. — Einhorn's saccharimeter.

Quantitative Estimation of Sugar.—Methods to be employed for the quantitative estimation of sugar are of necessity based upon the principles set forth in the qualitative tests previously outlined.

Fermentation Test.—This test, while sufficiently reliable to justify its use, is not practical for general laboratory work and is not, therefore, employed to the exclusion of Fehling's test. Neither can it be said to be absolutely reliable, though possibly it is slightly more so than is Fehling's reaction.

Take a quantity of the suspected urine, and render it acid by the addition of tartaric acid, after which it should be boiled for several minutes. When the urine is rich in glucose it is necessary to dilute it from four to ten or more times. The yeast to be employed must be fresh, and should have been tested in order to be certain that it possesses life. Rub up a portion of a cake of yeast (a piece about one-eighth inch square), mix it with the urine so as to form an emulsion free from lumps, and place the mixture in an Einhorn saccharimeter (Fig. 89).

Direction.—Pour the urine from the beaker into the saccharimeter until the expanded portion is nearly filled ; then place the thumb over the mouth of the apparatus and incline the tube so as to cause the urine-yeast mixture to fill the graduated portion completely, after which there will still remain some of the mixture in the expanded portion of the saccharimeter; and now add sufficient of the urine to fill the bulbous portion of the instrument. The saccharimeter and its contents should be set aside at a temperature of 25° to 35° C. (77° to 95° F.). As a guard against the escape of gas, a small amount of mercury may be poured into the bent limb of the saccharimeter. Should sugar be present, fermentation will occur within the course of twelve hours, the carbon dioxid formed collecting at the top of the tube, which as a consequence gradually displaces the urine as the gas accumulates. The percentage of sugar present is read from the graduation indicated on the perpendicular limb of the saccharimeter and this is to be multiplied by the degree of dilution.

Corroborative Test.—Introduce a small piece of caustic soda into the urine of the tube after the carbon dioxid is collected at its upper portion, and it will be seen that, owing to the absorption of gas, the liquid again rises toward the top of the tube.

Caution.—Yeast in itself may occasionally give rise to the formation of a small volume of gas, even in the absence of sugar, and it is therefore well to conduct control tests with normal urines. Among the other carbohydrates capable of fermentation should be mentioned lactose, levulose, and maltose, but should the tests with a copper solution prove positive, it is highly probable that glucose is present. None of the tests for sugar previously described is capable of demonstrating the minute traces present in normal urine, and the reader is referred for this purpose to special works upon physiologic chemistry.

Fehling's Method.—Fehling's solution (see Formula, page 227) is of such strength that the copper suspended in 10 c.c. will be completely reduced by 0.05 gm. of glucose. Urine containing sugar should be carefully added to this quantity of the solution until the copper is completely reduced, when the amount of sugar contained in the specimen of urine may be readily calculated. The desired results are most likely to be obtained when from 5 to 10 c.c. of urine are used in the titration, so that it is necessary to dilute the urine to the proper degree in the determination of this point, the specific gravity serving as a reliable guide (dilute five times, urines of specific gravity of 1.030), and to be more certain that the degree of dilution is sufficient treat 5 c.c. of Fehling's solution with 1 c.c. of diluted urine, a few drops of caustic-soda solution, and enough water to make 25 c.c. Boil the mixture thoroughly, and if the liquid still remains blue in color another cubic centimeter of dilute urine may be added and this procedure repeated until the two tests differ by 1 c.c. of urine—the last cubic centimeter that has been added causing the cuprous oxid to be separated. This precipitate, however, merely determines the approximate percentage of sugar present, and is unreliable unless all albumin has been previously removed from the urine.

Testing Fehling's Solution.—Dissolve 0.2375 gm. of crystallized cane-sugar, which has been dried at 100° C. (212° F.), in 40 c.c. of distilled water, and 22 drops of a one-tenth per cent. solution of sulphuric acid. Place the mixture on a water-bath and keep at boiling temperature for one hour; then cool and dilute to 100 c.c. with distilled water. Every 20 c.c. of this solution contains 0.05 gm. of glucose, which will reduce the copper in 10 c.c. of Fehling's solution should it be of the required strength. In case the solution be too strong, 21 c.c. or more of the sugar solution may be required completely to reduce the copper, in which case the strength of the Fehling's solution is determined according to the following equation:

$$20 : 0.05 :: 21 : x, \text{ making } x = 0.0525.$$

Titration.—Place 10 c.c. of Fehling's solution in a beaker and to it add 40 c.c. of water. Transfer to a porcelain dish or a flask and boil. While this mixture is boiling, add the dilute urine from a buret 0.5 c.c. at a time (Fig. 90), shaking after each addition, and it will be seen that the precipitated cuprous oxid will quickly settle at the bottom of the dish, forming a white precipitate, while simultaneously with this change the blue color of the reagent becomes less and less intense as the urine is added until finally the solution is nearly colorless. The urine should now be added drop by drop until decolorization is complete. Multiply the degree of dilution by five, and divide this result by the number of cubic centimeters of diluted urine employed. The quotient will indicate the percentage-amount of sugar.

In case of ordinary diabetic urine dilute 1 in 20 (5 c.c. of urine to 95 c.c. of water), and place a quantity of it in a buret. Place 10 c.c. of Fehling's solution in a flask of 100 c.c. capacity, add a few drops of caustic-soda solution, 40 c.c. of water, and boil. Add the urine from the buret to the boiling fluid until the liquid above the precipitate is decolorized, thus obtaining the approximate amount of urine required. Repeat the process in several flasks, adding the urine at once in each case until within one-tenth of a cubic centimeter of the amount of urine required to decompose the 10 c.c. of Fehling's solution has been found.

Computation.—Since 10 c.c. of Fehling's solution equal 0.05 gm. of glucose, let us suppose 10 c.c. of dilute urine were required and 5000 c.c. the product of twenty-four hours' secretion. The sugar contained in the twenty-four hours' urine is therefore $\frac{5000 \times 0.05}{10} = 25$ gm.; but since a dilution of 1 in 20 was employed in the twenty-four hours' urine, it equals 20 × 25 gm., or 500 gm.

Difficulties.—It is not at all times easy to determine the exact point at which all the copper has been reduced (as indicated by an entire disappearance of the blue color); but when it seems likely that this point has been reached, it is well to filter 1 c.c. or more of the fluid through Swedish filter-paper, acidify the filtrate with acetic acid, and treat with a few drops of potassium ferrocyanid. In the event of any copper being present in this solution a brown color will be formed, which indicates that the urine added is not sufficient; if, however, no browning of the liquid results, it is probable that the desired point has been reached and possibly passed. In either case repeat the titration for accuracy. Again in certain instances the precipitate will not collect at the bottom of the liquid and will even pass through the filter, thus rendering it impossible to determine the point of end-reaction (accumulation of reddish precipitate).

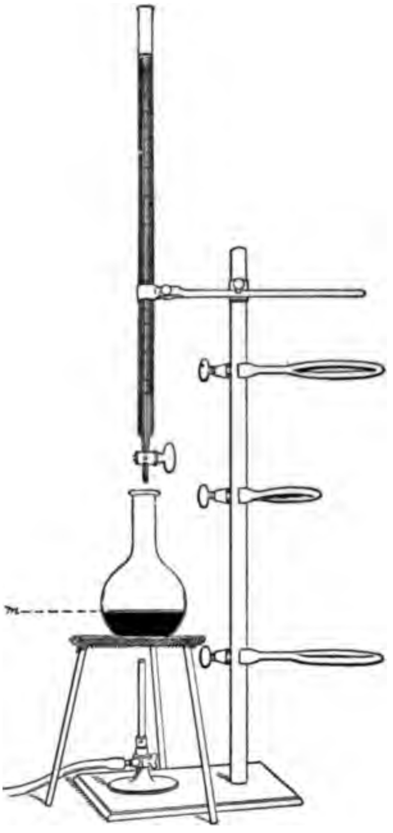


Fig. 90.—Apparatus for the quantitative estimation of sugar: *m*, Meniscus (Ogden).

Cause suggested the following method in such instances: Dilute 10 c.c. of Fehling's solution with 20 c.c. of distilled water and treat the mixture with 4 c.c. of a one-twentieth of one per cent. solution of potassium ferrocyanid. Boil, adding the dilute urine drop by drop from a buret until the solution is decolorized. No precipitate forms in this method.

Marshall and Ryan's Method.—Fehling's solution on a basis of the preparation of 1000 c.c. is made as follows: well-crystallized, non-effloresced, chemically pure cupric sulphate is pulverized and immediately spread on and pressed between sheets of bibulous paper to remove any moisture which may have been held mechanically by the crystals. Take 34.667 gm. of the pulverized dried material, quickly weigh, and dissolve in 400 c.c. of distilled water at ordinary temperature, and when complete solution has occurred, the liquid is diluted to 500 c.c. Agitate so that the solution shall become homogeneous in its contents of cupric sulphate. Place in a rubber-stoppered bottle.

Approximately 173 gm. of pulverized sodium and potassium (Rochelle salts) are dissolved in 480 c.c. of sodium-hydroxid solution of 1.140 specific gravity. After complete solution dilute with water to 500 c.c. Keep like the former solution.

When equal volumes of the cupric-sulphate solution and of the Rochelle-salt solution are mixed, Fehling's solution is produced and 1 c.c. of it will correspond to 0.005 gm. of glucose.

Dilute 1 c.c. of Fehling's solution with four volumes of water and heat to the boiling-point to ascertain whether decomposition of the solution has occurred. To the solution in the test-tube the urine is now added drop by drop from a pipet (noting the number of drops used), and after the addition of each drop of urine the liquid is heated to about the boiling temperature. Continue adding urine until the blue color of the liquid has disappeared. As two drops of urine are approximately equal to one-tenth c.c., therefore the number of drops of urine employed divided by two will furnish a number which, when expressed in tenths, will approximately correspond to the volume in tenths of a cubic centimeter of the urine employed.

The following table expresses, in tenths of a cubic centimeter, the percentage of glucose present in the urine as indicated by the quantity of the urine required exactly to decolorize 1 c.c. of Fehling's solution diluted with 4 c.c. of water:

CUBIC CENTIMETER OF URINE.	GLUCOSE PER CENT.	CUBIC CENTIMETER OF URINE.	GLUCOSE PER CENT.
0.1	5.0	0.4	1.25
0.12	4.2	0.45	1.1
0.14	3.5	0.5	1.0
0.16	3.1	0.6	0.83
0.18	2.7	0.7	0.71
0.2	2.5	0.8	0.62
0.25	2.0	0.9	0.55
0.3	1.66	1.0	0.5
0.35	1.4		

Instead of using the table the following rule may be employed: "Divide the whole number 5 by the number of tenths, converted into whole numbers, of urine employed. The result will be the approximate percentage of glucose."

Example.—If 12 drops of urine were required for the decolorization of 1 c.c. of Fehling's solution (two drops approximately equaling one-tenth c.c.), therefore 12 divided by 2 would give 6, which expressed in tenths would represent the number of tenths of a cubic centimeter corresponding to the drops of urine employed, namely, 0.6. This expressed as a whole number and used as a divisor of the number 5 $(5 \div 6) = 0.83$ per cent. of glucose.

To avoid computation, note the number of drops of urine necessary to decolorize 1 c.c. of Fehling's solution, and consult the following table for the approximate percentage of glucose:

DROPS.	GLUCOSE PER CENT.
1	
2	5.0
3	3.3
4	2.5
5	2.0
6	1.66
7	1.4
8	1.25
9	1.1
10	1.0
11	0.9
12	0.83
13	0.76
14	0.71
15	0.66
16	0.62
17	0.58
18	0.55
19	0.52
20	0.5
21	0.47
22	0.45
23	0.43
24	0.41
25	0.4

Differential Density Method.—Here, as in all estimations for sugar, albumin must be removed by heat and filtration and the urine should be sufficiently diluted with distilled water to insure that not more than 0.5 to 1 per cent. of sugar is present. The urine must be fresh, as fermentation begins spontaneously when the urine is allowed to stand. This method serves as a most valuable one in general work and is preferable to many of the more uncertain titration methods unless the operator has had considerable experience with such methods. Ascertain the specific gravity of the urine by means of a pyknometer or hydrometer, which should be graduated to the fourth decimal, and provided with a thermometer graduated to indicate tenths of a degree. (Each hydrometer is graduated to read at a certain temperature.) Take the specific gravity at the temperature for which the hydrometer has been constructed, heating or cooling the urine as may be found necessary.

Place 100 c.c. of the urine in a flask and to it add an emulsion of fresh live yeast which has been washed free from minerals. The flask should be corked loosely in order to allow the escape of gas, and the bottle set aside for from twenty-four to forty-eight hours, when again determine the specific gravity, observing the same precaution previously outlined. Multiply the difference in the specific gravity by 230, which factor has been found through estimations of sugar by titration, polarization, or by the different density methods of the urine after fermentation. This result is indicative of the percentage of sugar present. Whenever it is desired that this process be carried out within a shorter time, add to each 100 c.c. of urine 2 gm. of tartrate of potassium and sodium, with 2 gm. of diacid sodium phosphate and 10 gm. of compressed yeast, keeping the mixture at a temperature of from 30° to 40° C. (86° to 104° F.), when in a few hours fermentation will be completed. To the specific gravity obtained before fermentation should be added 0.022 to make up for the increase, owing to the addition of the salts, that will be observed in the second specimen.

Optical Saccharimetry.—Grape-sugar exercises a right rotatory power over polarized light, and on this account there is a method of both qualitative and quantitative estimation for sugar by the polariscope. If diabetic urine be light in color, and clear, it can be put into the glass tube of the instrument and a determination immediately made.

Caution.—In filling this glass tube observe that no air-bubbles are included with the fluid. But should it be dark and cloudy or contain albumin, it is necessary first to clarify the urine and remove the albumin and other disturbing substances.

Clarifying.—Urine may be clarified by adding to it a 10 per cent. aqueous solution of sugar of lead. The lead acetate causes a copious precipitate which consists of lead chlorids, phosphates, and sulphates, and in addition this precipitate carries down with it nearly all the coloring-matter and albuminous bodies present in the urine. The urine is now passed through a dry filter, and the filtrate is practically clear and ready for study. It is necessary to consider the degree of dilution caused through clarifying the urine. Take 75 c.c. of urine and to it add 25 c.c. of the lead solution; this dilution being observed in our calculation.

The Saccharimeter.—Elaborately constructed instruments for saccharimetry have been devised by a number of investigators, among which that of Ultzmann will be described; and with it rapid and fairly satisfactory results in the quantitative estimation of sugar may be obtained, should the quantity exceed 1 per cent., but where a smaller percentage of sugar is present, results are unreliable.

Artificial light is not needed, since the instrument is fitted to a microscope stand and the illumination accomplished by the mirror. The tube, objective, and ocular of the microscope are removed and in their place the saccharimeter is inserted and held in position by a small screw. The concave mirror is then adjusted and the instrument manipulated as the microscope (Fig. 91). *a* is a biconcave and *b* an objective lens of a small telescope, the focal distance of which extends to *p*; *c*, the upper Nicol prism with which the vernier is rather closely connected; *d*, a glass tube for holding the fluid to be examined; *p*, the upper plate of the right and left rotating quartz; and *f*, the lower Nicol prism. The arc or fixed scale is so divided that each division of it indicates 1 per cent. of grape-sugar when at a temperature of 20° C. (68° F.). The vernier enables one to estimate tenths of a degree of 1 per cent., since 10° C. (50° F.) of the vernier correspond with 9° of the arc. To the percentage of sugar found should be added as many tenths as there are spaces counted on the vernier—up to that division which coincides with a division of the arc. Should the zero point of the vernier not quite reach (toward the right) the 5-point of the scale, it indicates that the percentage of sugar exceeds 4 and is less than 5 per cent. When it is desired to estimate the tenths per cent. and the sixth division of the vernier is the first counted from the zero point to coincide with that division of the arc, six is the number of tenths required: hence the apparatus indicates 4.6 per cent. of grape-sugar present. The polarization of cane-sugar is three-fourths that of grape-sugar. Both cane- and grape-sugar, as well as lactose, possess the power to turn the polarized ray to the right—albumin and levulose, on the contrary, turn it toward the left.

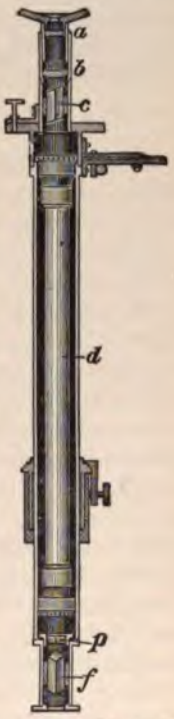


Fig. 91.—Sectional view of Ultzmann's polarizing saccharimeter.

In case the glass tube of the saccharimeter is empty or contains a fluid which holds in solution a substance having no optical influence, as has normal urine, the zero point of the vernier will be found to coincide with the zero point of the scale, and the halves of the field of vision are isochromatic. When an optically active substance is contained in the fluid (sugar), this normal isochromatism of the two halves is lost and an unequal coloring takes its place, and this inequality of coloring depends upon the amount of optically active substance contained in the solution. Whenever this unequal coloring is encountered, move the vernier toward the right or left, depending upon the substances present (sugar or albumin), until the color of the two halves becomes the same, and the percentage is then read from the graduated scale.

Computation.—Suppose to 75 c.c. of highly colored saccharine urine there have been added 25 c.c. of lead solution for clarifying purposes, and the filtrate is found by polarimetry to contain 4.8 per cent. of sugar. It is necessary to add to this percentage 1.6 per cent., since the lead solution added equaled one-third the bulk of the urine; therefore 6.4 per cent. equals the total amount of sugar present.

LACTOSE.

Lactose is to be suspected where a positive reaction is obtained with the copper and bismuth tests previously described, and negative results are obtained with the phenylhydrazin and fermentation tests; though it is to be remembered that osazone is to be obtained from the pure substances.

Clinical Significance.—Lactose is commonly found in the urine of nursing women where the flow of milk is impeded and a mastitis exists, and it is also encountered in nursing women with normally well-developed breasts and in wet-nurses. The percentage of lactose may fluctuate between 0.013 and 0.438 per cent.

PENTOSES.

Traces of pentoses—xylose, rhamnose, and arabinose—may be found in normal urine. Abnormally large quantities were observed by Salkowski in a morphin drunkard where pentosuria was found to alternate with glucosuria. Bial found two sisters and a brother to suffer from pentosuria. Cases have been observed where digestive pentosuria probably existed. Urines containing pentoses in rather large amounts reduce Fehling's solution and form osazone when treated with phenylhydrazin; but the fermentation test is negative. Rhamnose and arabinose possess the power of turning the plane of polarized light to the right; xylose remaining negative (see *Optical Saccharimetry*, page 236).

Bail's Test.—Mix one to one and one-half gm. orcin, 500 gm. of fuming HCl, and 25 to 30 drops of ferric-chlorid solution. Place 3 c.c. of the urine in a test-tube and to it add 5 c.c. of the reagent. Close the test-tube with a plug of cotton and heat over a flame until bubbles begin to form, when a green pigment is almost immediately deposited in the presence of pentoses. When a small amount is present, the pigment does not become perceptible until after fifteen to twenty seconds. This reaction is claimed to be negative with normal and with diabetic urines. It would appear questionable as to whether or not this test will differentiate glucosuric compounds from pentoses.

Tollen's Phloroglucin Test.—Place several cubic centimeters of phloroglucin in a test-tube, add one-half to one c.c. of the urine, and heat, when a deep-red color appears in the presence of pentoses. This reaction is also produced by glycuronates.

Clinical Import.—Maltose, levulose, dextrin, laiose, inosite, and animal gum may at times be found in the urine, but their clinical significance remains unsettled. While glycuronic acid is to be met with in the urine, it is of no known clinical importance.

BILE IN THE URINE.

Bile-pigment and also bile acids are to be met with in the urine and usually occur together, in which instance the pigment is much more abundant than are the acids. Obstruction of the bile-passages serves as a common cause for the entrance of the bile and its contents into the urine. It also remains a question whether or not traces of bile acids are present in normal urine. Biliary urine while fresh contains bilirubin, but after it has stood for a time biliverdin is formed as a result of oxidation. To the naked eye, urine containing bile is of a greenish or brownish-yellow hue, may at times be viscid, and upon shaking displays a heavy *froth* which partakes of the color of the urine. This froth serves to distinguish biliary urine from other urines known to display excessive froth when agitated; viz., albuminous urine (blood, pus, vaginal secretions, etc.), diabetic urine, urine rich in urates, calcium oxalate, phosphates, urine after the ingestion of salol, and occasionally those containing large amounts of other mineral products; but in none of these save urates does the froth partake of the color of the urine.

Tests for Bile.—1. Place a few drops of the urine in a porcelain dish, and at a point one-half to one inch distant from it place two or three drops of yellow nitric acid. By means of a glass rod draw the urine across the porcelain surface to meet the acid, when, upon their union, a display of colors immediately develops.

2. Place 50 c.c. of urine in a beaker and to it add 5 c.c. of a 10 per cent. solution of barium chlorid and 5 c.c. of chloroform. Shake well, and allow to stand for ten minutes. The precipitate which forms will be found to consist of phosphates, bile-pigment, and chloroform. Lift a portion of this precipitate in a pipet, place it on a shallow dish, and heat over a water-bath until the chloroform has evaporated, after which cool and pour off all

the fluid from the precipitate. The precipitate is now of a yellowish hue, and when treated with a drop of impure nitric acid directly upon the surface of the dish, the bile-pigment causes a display of colors to appear around the drop. This reaction reveals the presence of 0.2 per cent. or more of bile.

3. **Iodin Test.**—Place 10 c.c. of urine in a test-tube and allow to flow upon it, entering along the side of the tube, a 10 per cent. alcoholic solution of iodin. At the zone of contact of the liquids an emerald-green layer appears and is due to the presence of bile.

Test for Bile Acids.—Oliver has proposed a test which depends upon the power of the bile acid to precipitate peptone when in acid solution.

Reagent.—

℞.	Powdered peptone	½ dram
	Salicylic acid	4 grains
	Acetic acid	½ grain
	Distilled water	8 ounces.

Place 20 minims of clear filtered urine in a test-tube and to it add 60 minims of the reagent. In the presence of bile acids a decided milkiness at once appears, and should the acids be large in amount, this turbidity is most apparent. Agitation often causes it to disappear, but it readily reappears on the addition of the reagent. This test is exceedingly delicate and thus far is believed to be one of the most reliable for the detection of bile acids in the urine.

ACETONE.

Hydroxybutyric acid, aceto-acetic acid, and acetone are all to be met with in the urine, and the relationship existing between these three substances is explained by the following formulæ:

Hydroxybutyric acid = $CH_3CH(OH)CH_2COOH$.
Aceto-acetic acid = CH_3CO,CH_2COOH.
Acetone = CH_3,CO,CH_3.

Hydroxybutyric acid is the first to be formed from the destruction of proteids (probably), and after oxidation yields aceto-acetic acid, and in turn aceto-acetic acid is readily decomposed into acetone and CO_2.

Test for Aceto-acetic Acid.—Fill a test-tube one-third its depth with fresh urine, and to it add a few drops of a solution of perchlorid of iron (diluted to a pale cherry color) until the precipitate of phosphate and iron ceases to fall; filter, and to the filtrate add another drop or more of the iron solution. In the presence of aceto-acetic acid a claret color develops which disappears upon boiling. Salicylates, carbolic acid, antipyrin, and similar drugs cause a claret color with perchlorid of iron, and are to be differentiated from that produced by aceto-acetic acid by the fact that the color induced by these drugs does not disappear on boiling.

Tests for Acetone.—I. (*a*) Acetone urine emits a fruit-like odor.

(*b*) Reduces Fehling's solution, and the test which serves most readily to identify this substance is to convert it into iodoform.

II. Place about one inch of urine in a test-tube and to it add five drops of a 10 per cent. solution of caustic soda or potash; heat gradually and add, drop by drop, a saturated solution of iodin in potassium iodid until the liquid assumes a yellowish-brown color. Again, add a few drops of caustic-soda solution. A yellow turbidity ensues which soon collects at the bottom in the form of a crystalline precipitate emits the odor of iodoform. Microscopically this precipitate consists of hexagonal plates which are at times in rather dense aggregations and in the form of stars.

In case there is but a trace of acetone present it will be found necessary to distil the urine after the addition of phosphoric acid, and then test the distillate as above described.

Clinical Significance.—Acetone under normal conditions may appear as a mere trace in the urine, at which time the amount excreted during the twenty-four hours may vary from 0.008 to 0.025 gm.

Diet.—The quantity of acetone excreted is influenced by the character of food ingested; *e. g.*, the withdrawal of carbohydrates causes a decided increase which reaches its maximum point in from five to eight days, at which time 100 to even 700 mgm. may be excreted daily. Such examples of acetonuria disappear where carbohydrates are given with the food. Acetonuria is most decided when the diet has contained the minimum amount of albumin and no carbohydrates. It is also marked during starvation.

Acetonuria is a feature of diabetes, wasting diseases, and of both acute and prolonged febrile conditions.

Diabetes.—The appearance of acetone in the urine of diabetics is of great clinical significance, and when acetone and sugar are both present, the amount of the former is in rather direct correlation with the severity of the disease. With a gradual daily increase in the amount of acetone a fatal issue is to be expected at an early date. As previously stated, the addition of carbohydrates to the food may lessen greatly the daily excretion of acetone, and it is questionable whether or not the approach of diabetic coma may be delayed through the administration of carbohydrates.

Fevers.—Here should be mentioned pneumonia, typhoid fever, acute articular rheumatism, miliary tuberculosis, septicemia, scarlet fever, measles, tonsillitis; as well as phthisis with cavity formation and other intermittent fevers.

Caution.—In continued fevers the large amount of acetone excreted through the urine is dependent in part, at least, upon the fact that such patients are not fed liberally upon carbohydrates and albumins.

Nervous Diseases.—Acetonuria is also observed in such nervous conditions as melancholia, general paresis of the insane, major epilepsy, locomotor ataxia; and where malnutrition is in evidence.

Gastric Carcinoma.—An exception to general loss of flesh as an accompanying feature of acetonuria is the fact that acetonuria is likely to obtain early during the course of gastric carcinoma, and even before emaciation is apparent.

Chloroform.—Chloroform anesthesia is often followed by acetonuria.

It is generally conceded that acetonuria may result from certain gastro-intestinal derangements (*enterogenic acetonuria*); and it is probable through this manner that asthma is associated with acetonuria. Acetonuria further suggests that there is a general breaking down of the circulating albumins.

OXYBUTYRIC ACID.

Detection.—Oxybutyric acid is detected, or at least suspected to be present, in diabetic urine when the urine, after subjection to fermentation, rotates the plane of polarized light to the left (see *Optical Saccharimetry*, page 236).

Caution.—Albumin also exercises a rotatory power and must needs be removed by boiling and filtration. Decolorize highly colored urines.

Clinical Significance.—The presence of oxybutyric acid in the urine is of special clinical moment in connection with diabetes mellitus, occurring only in severe forms of this disease. The quantity of oxybutyric acid present is usually in direct relation to the amount of sugar excreted. Oxybutyric acid is omnipresent in the urine of diabetic coma, and is believed to contribute toward the auto-intoxication now conceded to be responsible for such coma. Stadelmann has suggested that the acid exercises a toxic effect by absorbing alkali from the blood.

Oxybutyric acid has been found in the urine during the course of acute exanthematous conditions and in scurvy. It may also be found during insanity. The relation existing between the excretion of oxybutyric acid and of ammonia is of valuable clinical importance (see *Ammonia*, page 246).

DIACETIC ACID.

Diacetic acid is not a constituent of normal urine, and when found present in this secretion is of practical clinical importance. There is, as a rule, a co-appearance of oxybutyric acid, acetone, and diacetic acid in the same urine. Diacetic acid is a product of the further oxidation of oxybutyric acid (Vierordt), and eventually diacetic acid is decomposed into acetone and carbonic acid.

Gerhardt's Test.—1. Diacetic acid is detected in the urine by treating 10 c.c. of the suspected specimen with a solution of perchlorid of iron, which should be added drop by drop. The addition of the iron solution may cause a precipitate of phosphates, which sediment is removed by filtration. Treat the filtrate further with the iron solution, and should diacetic acid be present, a Burgundy-red color develops, which may deepen to a dark-brown.

2. Boil a second quantity of the urine and treat as in the case of No. 1. In the event of no reaction taking place, proceed as follows:

3. Place 10 c.c. of the urine in a test-tube; add a small quantity of sulphuric acid, and extract with ether. Treat this ethereal extract with the perchlorid of iron solution as outlined above, when the red color will develop. Upon standing for twenty-four hours the red color will be seen to fade, and eventually to disappear, should such color be due to the presence of diacetic acid, "if acetone is present at the same time" (von Jaksch).

Arnold's Test.—*Reagents.*—(*a*) A solution of paramido-acetophenone: dissolve one gm. of paramido-acetophenone in 100 c.c. of distilled water. Add to the solution hydrochloric acid, one drop at a time, until the yellow color disappears, care being taken that no more hydrochloric acid be added than is absolutely necessary to cause a colorless solution. (*b*) A solution of sodium nitrite (1 per cent.).

Method.—1. At the time of use place 6 c.c. of solution (*a*) in a test-tube, and to it add 3 c.c. of solution (*b*).

2. Add an equal volume of filtered urine (filter highly colored urine through animal charcoal), and follow with a few drops of ammonia.

3. With this solution, normal urine will be found to show a variable degree of color, which becomes brown or brownish red after the mixture is thoroughly shaken. In the presence of diacetic acid a reddish-brown amorphous precipitate collects at the bottom of the fluid after the tube has been allowed to stand for a short time.

4. Place 1 c.c. of the supernatant colored liquid in a dish, and to it add 10 c.c. of concentrated hydrochloric acid. Diacetic acid when present in appreciable amount causes a purplish-violet color.

It is claimed for Arnold's test that it does not react with acetone, oxybutyric acid, bilirubin, and after the administration of antipyretics, salicylic acid, etc.

Clinical Significance.—Diacetic acid may be found in the urine in diabetes mellitus, where it may exist for a long period of time without coma, and during the course of the acute fevers, including typhoid, scarlet fever, pneumonia, articular rheumatism, tonsillitis, etc. Diaceturia may also occur during febrile conditions of an intermittent type. Again, diacetic acid is a not infrequent constituent of the urine of fevers in children, and is to be found in the urine during the course of gastro-intestinal derangements.

Diacetic acid is commonly present in conjunction with acetone, and is always of serious moment when found in the urine of adults. "If diaceturia is present, oxybutyric acid is also present in the urine" (Vierordt). Von Jaksch has suggested that the convulsions occurring during acute febrile diseases in children are accompanied by diaceturia.

LACTIC ACID.

Lactic acid (or sarcolactic, as it sometimes occurs) is not a constituent of normal urine, but it may rarely be observed during disease.

Reagents.—(*a*) Alcohol 95 per cent.

(*b*) A dilute solution of sulphuric acid.

(*c*) Sulphuric ether.

(*d*) A 1 per cent. solution of basic acetate of lead.

(*e*) Zinc carbonate.

Method.—1. Place a few cubic centimeters of the suspected urine in a dish, and evaporate over a water-bath to a syrupy consistence; then extract with 95 per cent. alcohol.

2. At the end of twenty-four hours remove the supernatant alcohol, and again evaporate the mixture to a consistence of syrup; after which acidulate with a weak solution of sulphuric acid.

3. The mixture is now extracted with ether, continuing the process as long as the mixture displays an acid reaction.

4. Remove the ether by distillation, and dissolve the residue in a small quantity of water.

5. Place the watery mixture in a test-tube, and to it add, drop by drop, a small quantity of the lead solution. The mixture is now filtered, and the lead that is yet remaining removed with sulphureted hydrogen.

6. Place the filtrate in a dish, and evaporate to dryness over a water-bath, the faintly yellowish, sticky residue being lactic acid.

7. Dissolve the residue in a small quantity of water, and heat the mixture with zinc carbonate until it is saturated, when upon evaporation zinc lactate will be found to separate in the form of small crystalline prisms.

Clinical Significance.—Sarcolactic acid is a constituent of the urine in certain hepatic maladies.

CHOLESTERIN.

Crystals of cholesterin (Fig. 92) are rarely met with in urinary sediments. Von Jaksch detected cholesterin in tabes dorsalis and in cystitis; Simon found it in acute nephritis, and I have encountered it upon three occasions—two of the urines were recovered from cases of chronic cystitis, and in the third an echinococcic cyst had ruptured into the urinary tract. The fresh urine containing cholesterin displays an acid reaction, is usually turbid, and when shaken vigorously, numerous white, flaky scales are to be seen throughout the liquid. Cholesterin is commonly found in the fluid recovered from cysts and in feces (see *Tests for*, page 370).

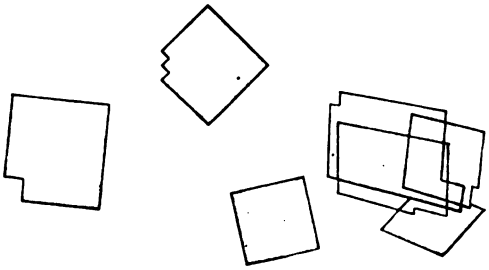


Fig. 92.—Cholesterin crystals (Ogden).

AMMONIA IN THE URINE.

Ammonia is a constituent of normal urine, from 0.05 to 0.08 gm. being excreted daily. The quantity of ammonia will be found greatly increased when the urine contains oxybutyric acid, such increase bearing a direct relation to the amount of oxybutyric acid present. During the course of diabetes mellitus it is common to find from 2 to 6 gm. of ammonia excreted during the twenty-four hours, and such patients are in imminent danger of developing diabetic coma. At this time the urine is usually rich in acetone, oxybutyric acid, and diacetic acid. (See *Urea*, page 192.)

CYSTIN.

Cystin is occasionally found to form one of the deposits of acid urine, and its recognition depends upon the microscopic characteristics of its crystals. Cystin precipitates disappear from alkaline urines since they are soluble in alkalis, but such urines are likely to putrefy and give off an odor of sulphureted hydrogen.

Tests.—Place 10 c.c. of urine in a test-tube and to it add 1 c.c. of a 30 per cent. solution of caustic potash. Boil, and while boiling add, drop by drop, a solution of acetate of lead (2 to 10 per cent.). A black precipitate of lead sulphid develops.

Clinical Significance.—Cystin is far more common in the urine of males than in that of females, and it is rarely to be met with in the aged. Several cases have been reported as occurring in members of the same family. Cystinuria may be persistent or intermittent. It occurs in the urine where there exists a cystin calculus.

PYURIA.

Urines containing pus usually display a rather heavy whitish or creamy precipitate which forms as a solid sheet at the bottom of the liquid, and when the bottle is shaken, the sediment becomes ropy or shred-like. Pus may occur in the urine in conjunction with blood or with hemoglobin, in which case the naked-eye appearance of the precipitate (dark) is somewhat misleading. Urines containing pus are likely to be rich in albumin, and a point of special interest is to determine whether or not all the albumin present results from the pus, or whether true renal albuminuria exists. Reinecke suggested the collecting of the twenty-four hours' urine, shaking it thoroughly and then estimating the number of pus-cells by the hemocytometer. He believes that 100,000 pus-cells per c.mm. should correspond to 1 per cent. of albumin, as shown by Esbach's albuminometer. Taking into consideration the many factors that may interfere seriously with the dilution of the urine and consequently render our count valueless, this procedure seems hardly worthy of recommendation. It is probably far better to endeavor to eliminate albumin originating along the urinary tract by filtration, since renal albumin is thus separated from the urine with great difficulty.

Tests for Pus.—Urines which contain pus develop a green color on the addition of a solution of guaiac, which disappears when the mixture is heated. Add liquor potassium to the deposit of pus and the sediment becomes ropy and later forms into a gelatinous mass. The best evidence of pus in the urine is the detection of pus-corpuscles (cells) by the microscope.

Clinical Significance.—The urine may contain pus whenever there is a suppurative process either involving any portion of the genito-urinary tract, or where pus may escape into the tract through a fistulous opening. Pus may also have its origin in the vagina or cervix, and true pyuria should be distinguished from this condition, which simulates pyuria of urinary origin closely, by catheterization of the patient.

ALKAPTONURIA.

Alkaptonuria is a condition wherein the urine presents the natural color when voided, but after exposure to the air and light, it becomes gradually dark on the surface, which darkening extends toward the bottom of the liquid and eventually becomes a reddish brown, dark brown, or black. This color is dependent upon the presence in such urines of dihydroxyphenylacetic acid. Melanin and also phenol or its derivatives, when present in the urine, cause it to become dark after exposure.

When alkapton is treated with a small amount of alkali, the color appears much earlier, and in fact almost immediately; and upon application of heat Fehling's solution is reduced by such urines. It is to be distinguished from glucosuria, however, by the fact that negative results are obtained with the bismuth test (page 229). With an ammoniacal silver solution reduction occurs without heat; and a bluish-green color develops (temporarily) when the urine is treated with a ferric salt. The fermentation test, phenylhydrazin test, and polarimetry are also negative.

Garrod's Test.—Heat 100 c.c. of the fresh urine to near the boiling-point and add 5 or 6 gm. of solid neutral lead acetate. Upon dissolution of the lead a bulky grayish precipitate forms, which is removed by filtration. The filtrate (pale yellow) should be set in a cool room for twenty-four hours. Should the urine be rich in homogentisinic acid, minute, acicular, almost colorless crystals begin to form after several hours, and in case the flask containing the urine be set upon ice, crystallization is hastened. These crystals are grouped to form rosets and stars and are rather deeply colored. Should crystallization be tardy, again heat the urine and add more of the lead acetate. The crystalline product thus obtained is lead homogentisinate, and is soluble in hot water, but upon its solution the liquid becomes a deep brown when an alkali has been added. It reduces Fehling's solution and causes a deep-blue color with a dilute solution of ferric chlorid.

Clinical Significance.—Alkapton may appear spontaneously in the urine of apparently healthy individuals. Garrod[*] states that the condition is to be met with in several members of a family, and cites cases demonstrating this point. It affects brothers and sisters alike, but has not been shown to be transmissible from one generation to another. Intermarriage exercises some influence favoring the development of this condition, and older members of the family are less liable than are the younger ones. Pavy has observed a family in which the parents were first cousins, where four of fourteen children were alkaptonuric. Mittlebach[†] reports an interesting case in conjunction with a chemic analysis of the substance. The amount of homogentisinic acid eliminated in twenty-four hours is variable and may reach 4.6 gm. An increased excretion follows the excessive ingestion of meats.

UREIN.

Moor,[‡] of Rome, Italy, claims to have discovered a substance in the urine which is probably one of the most valuable contributions to recent urinology should it stand the test of criticism. Urein is described as being an oily liquid, of heavy specific gravity, and of a pale yellow color, resembling olive oil. Weatherson[§] has contributed a valuable monograph upon the chemistry of this substance. In 50 c.c. of urine nearly two ounces of liquid urein are claimed to exist, though the latter observer was unable to recover this quantity from the twenty-four hours' product. Urein remains intact and constant at a temperature below 75° to 80° C. (167° to 176° F.), yet further thorough and careful investigation needs to be made by other observers.

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[*] "Lancet," Nov. 30, 1901.

[†] "Deut. Archiv f. klin. Med.," vol. lxxi, pt. i.

[‡] "Proc. Inter. Med. Con.," Paris, 1900.

[§] "Jour. Amer. Med. Ass.," March 16, 1901, p. 723.

EHRLICH DIAZO-REACTION.

Under certain pathologic conditions associated with high fever a chromogen is to be found present in the urine, which, when treated with diazobenzinsulphonic acid in the presence of ammonia, causes a distinct red color which may vary from a rose-pink to a garnet. The reaction probably depends upon the fact that if sulphanilic acid (amidosulphobenzol) be acted upon by nitrous acid, diazosulphobenzol results, and unites with the aromatic compounds present in the urine, forming an anilin color.

Reagents.—1. A solution of sulphanilic acid (1 gm. to every 100 c.c.) in 5 per cent. hydrochloric acid.

2. Solution of sodium nitrite one-half per cent. It is necessary that both solutions be fresh.

Method.—Place 10 to 20 c.c. of urine in a test-tube and to it add an equal volume of solution No. 1, shaking gently to effect a perfect mixture; then add from three to six drops of solution No. 2, and shake until a heavy froth collects. Render alkaline with ammonia. The diazo-reaction consists in the liquid becoming a port-wine color while the froth is also red.

Clinical Significance.—The diazo-reaction is a fairly constant symptom of typhoid fever after the second week of the disease. I have found it present repeatedly in cases of measles, tuberculosis (with cavity), meningitis, croupous pneumonia, in a number of rather obscure conditions, the majority of which were associated with high fever; and less often in scarlet fever, acute miliary tuberculosis, erysipelas, pyemia, diphtheria, puerperal sepsis, and tonsillitis. It has not, therefore, proved to be of great diagnostic value in typhoid fever, since the disease is usually capable of diagnosis before the urine displays this reaction, though certain of its advocates claim that the reaction is to be found as early as the fifth day of the disease. In pulmonary tuberculosis where the diazo-reaction is present for a long period the disease usually progresses with great rapidity.

EHRLICH'S DIMETHYLAMIDOBENZALDEHYD REACTION.

It remained for Ehrlich * to point out that under certain conditions dimethylamidobenzaldehyd induced a red color with mucus of some pathologic urines (which are always of high specific gravity—1.026 to 1.034), and that a reaction resembling this, though less intense, was also given with normal urines. Pathologic urines giving this reaction are also likely to give positive diazo- and indican reactions. Proscher's analytic results are:

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* "Med. Woch.," 1901, No. 15.

carbon 56.49; hydrogen 7.06, and nitrogen 8.28 per cent., and he gives the formula $C_7H_{15}N$ for this unknown body.

Method.—*Reagent.*—Place 2 per cent. of dimethylamidobenzaldehyd in equal parts of concentrated hydrochloric acid and water. Place 10 to 20 c.c. of the urine in a test-tube, and to it add from five to ten drops of the reagent; agitate to effect a mixture and set aside for a few minutes (two to four) and then note the color. The reaction is positive when a cherry-red color develops and takes place with certain pathologic urines. Normal urines may show a deepening of their normal color, redden slightly, or change to a greenish yellow. Even with pathologic urines the intensity of the red color is variable. It may develop upon contact of the first drop of the reagent with the urine; or it may require several drops of the reagent, and two to four minutes' exposure before the maximum intensity is attained. The pigment can be extracted in part with chloroform.

Caution.—After standing for five minutes the cherry-red color is apt to give way to a dark amber, yet rarely, with a decided reaction, the color may persist for hours and even days.

Clinical Significance.—Positive results may be obtained with the urine of tuberculosis in 40 per cent. of cases. The clinical reports of Clemens,[*] Koziczkowsky,[†] and Simon [‡] show that the reaction is also common to the urine of both febrile and non-febrile maladies, among which are to be mentioned gastro-enteritis, alcoholism, appendicitis, chronic plumbism, gastric carcinoma, esophageal carcinoma, lobar pneumonia, bronchitis, subphrenic abscess, facial erysipelas, measles, and typhoid fever.

THE VOLATILE FATTY ACIDS.

Volatile fatty acids when present in the urine are usually described under the caption of lipaciduria, although the term, more correctly speaking, implies an increased elimination of this substance. The fatty acids which have been found in the urine are acetic, butyric, propionic, and formic acids.

Detection.—1. The detection of fatty acids in the urine is accomplished by distilling the urine with phosphoric acid; the distillate cautiously neutralized with carbonate of soda, and evaporated to dryness over a water-bath.

2. Extract the residue with hot alcohol; filter, and again evaporate to dryness over a water-bath.

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[*] "Deut. Arch.," 1901, vol. lxxxi, p. 168.

[†] "Berl. med. Woch.," 1902, vol. xxxix, No. 44.

[‡] "Amer. Jour. Med. Sci.," Sept., 1903, p. 471.

3. Dissolve this second residue in water, and to this liquid apply a small quantity of a solution of perchlorid of iron (the solution of formic acid must be neutral in reaction), when a blood-red color develops. Heat this solution gradually to the boiling-point, when the color, should it be due to formic acid, fades and eventually disappears, leaving the deposit of a rusty colored sediment at the bottom of the liquid (see *Feces*).

Place 10 c.c. of the urine in a test-tube, and to it add one-fourth this volume of sulphuric acid and alcohol, when, in the presence of acetic acid, an odor of acetic ether is emitted.

It is important to note that free formic acid is not precipitated by nitrate of silver; but that the alkaline salts of formic acid are precipitated from concentrated solutions containing this substance by nitrate of silver; a white precipitate results which soon changes to a black should formic acid be present.

Microscopically, volatile fatty acids may appear in the form of needle-like crystals (see *Feces*).

Clinical Significance.—Normal urine contains traces of the fatty acids. Fatty acids are known to occur in excessive amount in the urine during the course of leukemia, diabetes, cirrhosis of the liver, and in suppurative conditions, among which should be mentioned those affecting the serous sacs, *e. g.*, purulent peritonitis, pleurisy, etc.; and in such acute inflammatory maladies as erysipelas and phlegmonous inflammations.

It is difficult to estimate the true clinical significance of fatty acids when found in the urine, and while it is generally conceded that the degree of carbohydrate fermentation present in the intestine bears a more or less direct correlation to the amount of fatty acids excreted through the urine, further evidence is needed to substantiate this belief.

FAT.

Fat is not a constituent of normal urine, but during the course of certain maladies it may appear in this secretion in rather large quantities. This condition is known as *lipuria.*

The appearance of sufficient fat in the urine to render it of a suspicious appearance is unusual, although there are valid records of such instances, where the urine has been found to contain fats during the course of what otherwise appeared to be normal pregnancy. It is all-important to exclude the possibility of the urine being contaminated with fat from structures adjacent to the urinary tract. Microscopically it is not unusual to detect small particles of fat in the urine.

Clinical Significance.—Small fat-globules are to be observed microscopically whenever there exists advanced degeneration of the renal epithelium. Areas of fatty degeneration are to be recognized in such epithelial cells and in the pus-corpuscles. Under such conditions droplets of fat may be found free in the urine, but it is far the more common to find them intimately connected with, or apparently occupying, a portion of some of the formed elements (see *Fatty Casts*, page 289). Lipuria may be preceded by lipemia (see *Fat in the Blood*, page 66).

Certain observers have found varying amounts of fat in the urine after the administration of large quantities of cod-liver oil. Lipuria is a feature of obesity, pulmonary tuberculosis (stage of cavity), leukemia, disease of the pancreas, myocarditis, diabetes mellitus, chronic nephritis with fatty kidney, phosphorus-poisoning, pyonephrosis, puerperal eclampsia, chronic alcoholism, and conditions in which abscesses and cysts empty into the urinary tract. It has been observed to follow multiple fractures of the long bones, and in septicemia.

Staining.—Place a small amount of the urinary sediment upon the center of a slide, spread thinly, and dry in the air. Stain for one minute with an alcoholic solution of Sudan III. Remove the stain by allowing a current of water to flow upon one end of the slide and to flood gently over the specimen. All particles of fat will stain pink or red with this solution. It is ofttimes more practical to place a drop of the Sudan III solution at the margin of the cover-glass, and to move the cover slightly toward the stain; when the red solution will be found to flow beneath the cover, mingling with the cellular elements and débris present. Thus treated, particles of fat stain a light pink shade.

For a description of Cammidge's reaction for glycerin in the urine see p. 531.

CHYLURIA.

Chyluria is the name applied to a condition wherein the urine presents the macroscopic appearance of milk. Chylous urine is likely to contain albumin, and always contains fat. Upon standing, such urine may display a heavy sediment, a portion of which sinks to the bottom, while a certain amount of the fat may collect upon the surface of the urine, resembling the cream displayed by milk that has been kept at a low temperature. Further, chylous urine may, upon standing, show a tendency toward the formation of rather tough coagulum. A portion, and at times nearly all, of the urine becomes gelatinized. Chylous urine often has a bloody color.

Recognition.—Place 10 c.c. of the milky urine in a test-tube, and to it add an equal volume of sulphuric ether; then shake vigorously for several minutes; and set aside for a short time, when the particles of fat are seen to be separated by the ether.

Chemically, fats form a rather constant constituent, and are to be recognized through both their chemic and microchemic reactions. Albumin also forms a common constituent of chylous urine, and is not infrequently present where there exists no other evidence of disease of the renal parenchyma. Such albumin may result in part at least from the blood that is present in chylous urine, but in the absence of blood and other evidence of renal disease it is often found present, in which instance its significance and derivation remain moot.

Microscopic.—The sediment of chylous urine is best collected through the aid of the centrifuge. Such sediment will be found to contain for the most part innumerable small refractile globules of fat (Fig. 93), epithelial cells, few leukocytes, and a variable number of red blood-cells, which in bloody urine may exceed in number the fat-globules. Crystals of leucin and tyrosin are occasional findings, while cholesterin is rather commonly seen.

Renal epithelium and renal casts are to be seen whenever there exists nephritis. It is not uncommon to find the Filaria sanguinis hominis present in chylous urine—a condition which I have observed upon two occasions. The filaria when passed with the urine is non-motile, but in fresh specimens the parasite does not appear to be materially distorted.

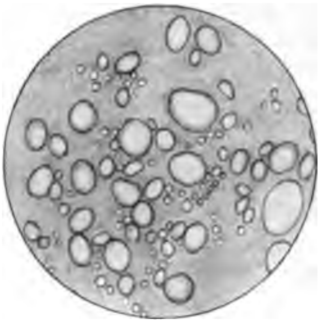

Fig. 93.—Chyle and starch in urine. Small cells chyle, large and irregular bodies are starch (obj. Spencer one-sixth).

Clinical Significance.—Parasitic chyluria is a symptom of filariasis, but by no means do all cases of filariasis show chyluria. In many instances of chyluria the true cause of this symptom is unknown; yet when chyluria is considered with reference to the relation of the lymphatics surrounding the kidney, it appears reasonable that any condition capable of forming a fistulous communication between the lymphatics and the pelvis of the kidney might excite chyluria. In one instance under personal observation chyluria followed an operation for a floating kidney; and in another a temporary chyluria developed following a severe fall, but this symptom (chyluria) was of but two days' duration. In a third case, recently studied, chyluria developed during the course of a sloughing uterine fibroid, but the urine became clear three days later and there has been no return of this symptom. It is of further clinical interest to note that this patient developed phlegmasia alba dolens two months after the attack of chyluria.

PTOMAINS.

Despite the vast amount of literature that has recently accumulated with reference to the detection of ptomains in the urine, we are thus far practically without clinical data that may be regarded of diagnostic value concerning such substances.

Cadaverin and putrescin have been found in the urine in considerable amounts. These substances are to be obtained from the cadaver during the process of putrefaction. They appear in the rice-water stools of Asiatic cholera and in cultures of the comma bacillus, of Koch. Cadaverin is often found in conjunction with cystinuria, and according to Simon, the amount of diamins found in this condition may vary greatly, he having isolated as much as 1.6 gm. of benzoylated cadaverin from the twenty-four hours' product.

GASES.

Normal urine contains a small volume of gases, which according to Purdy are: carbonic acid 4 to 9 volumes free gas, 2 to 5 combined; oxygen, 0.2 to 0.6 volume; and nitrogen, 0.7 to 0.8 volume.

Sulphureted Hydrogen.—Under certain pathologic conditions sulphureted hydrogen is found in the urine—a condition known by the term hydrothionuria. Hydrothionuria may be due to the escape of gas into the bladder from the adjacent tissues or viscera. In certain instances it has been found to be due to an accumulation of pus; and where abscesses have emptied their contents directly into the bladder. Again, sulphureted hydrogen is a product of the putrefaction of albuminous substances, in which case the gas is, at times, due to the development of bacteria. In order to prove this point, add a small quantity of the contaminated urine to several times its own volume of normal urine; mix thoroughly, place in a sterile test-tube, cork with cotton, and allow to stand for twenty-four hours at a temperature near that of the body. Should the gas be due to bacterial development, the normal urine in the test-tube will contain sulphureted hydrogen; but should the sulphureted hydrogen have gained entrance to the bladder from without, the urine will be found free from this gas.

It is not definitely known through the development of what organism or organisms sulphureted hydrogen is generated in the urine, and there is reason to suspect at least that it results from the action of certain bacteria upon some special substance or substances present in the urine. In addition to the above sources of sulphureted hydrogen in hydrothionuria, it is thought that the gas may be derived from neutral and oxidized sulphurs present. From the foregoing statements it will be seen that the entire question remains unsettled. Hydrothionuria is often associated with cystinuria.

Test for Sulphureted Hydrogen.—1. Place from 10 to 30 c.c. of the suspected urine in a clean bottle.

2. Cut several strips of filter-paper, about one-fourth inch wide by one inch long, and moisten these papers with a solution of sodium hydrate, and then with a solution of lead acetate.

3. Surround the moistened strips of filter-paper by a thread, and then lower them into the bottle, clamping the thread with the cork, so that the filter-paper will be suspended some distance above the urine, and near the top of the bottle.

Should sulphureted hydrogen be present, the filter-paper will be changed to a gray, brown, or black color. The intensity of the color and the time necessary for its production will depend upon the quantity of sulphureted hydrogen present.

EFFECT OF DRUGS UPON THE URINE.

It is the rule for clinicians to submit specimens of urine for analysis without informing the laboratory worker as to the nature of the case in question, and much less to think of acquainting him with what drugs have been administered to the patient during the past few days. Certain medicines are readily detected in the urine, while others are detected with great difficulty, and still a third class are not demonstrable except when administered in large doses, and even then there may be room for question as to their identity. It shall be my purpose here to call attention to a few drugs which when administered by the mouth produce decided changes in the urine.

Clinical Significance.—The recognition of certain drugs in the urine is by no means a feature of great clinical value, since drugs when given as medicinal, and even in toxic, doses are often eliminated with the urine in such small amounts as to make their detection impossible or at least impracticable.

Iodin.—Place 5 c.c. of urine in a test-tube and to it add from two to five drops of fuming nitric acid. Add chloroform to equal the quantity of urine, shake gently, and set aside for a few minutes. Chloroform will be found to settle at the bottom of the tube and display a reddish-violet color.

Bromin.—Bromin when present in the urine may be detected in the same method as is iodin, differing only in that bromin colors the chloroform a brownish yellow.

Potassium iodid gives a pink color to the chloroform when treated as in testing for indican (page 211).

Salicylic Acid and the Salicylates.—Following the administration of salicylic acid, the urine will be found to strike a blue-violet color when treated with a solution of chlorid of iron (see *Diacetic Acid*, page 243).

Salol.—Urine containing salol becomes green, and eventually black, upon staining (see *Color*, page 169). It gives the reactions for salicylic acid.

Antifebrin.—This substance may be detected in the urine by the following method: Place a few cubic centimeters of the fresh urine in a test-tube and to it add one-fourth this volume of a concentrated solution of hydrochloric acid; boil gently for from one to three minutes, and set aside until cool. To the cold mixture add a few cubic centimeters of a weak solution of carbolic acid (3 per cent.), and follow by adding, a drop at a time, a weak solution of chromic acid. The entire mixture assumes a red color; but upon being alkalinized by the addition of ammonia it changes to a blue. ***Antipyrin*** also causes a red color when treated with a solution of chlorid of iron; and ***thallin*** causes a greenish-brown color with this reagent.

Rhubarb and Senna.—Rhubarb and senna are known to give a decided color to the urine. These substances have been described under *Color*, page 169.

Carbolic Acid.—Following the administration of carbolic acid, resorcin, and naphthalin, the urine will be found to contain hydroquinon in considerable amounts; and upon standing exposed to the light, such urines darken, becoming first olive-green, and later brownish black, or in some instances black. The exact determination as to which of these drugs has been administered is of no practical value except in the case of poisoning (see special works on toxicology).

Poisons.—Whenever arsenic, antimony, lead, mercury, and silver have been administered in sufficient quantities to produce poisonous effects, these metals will be found to appear in the urine. They are also found after the prolonged use of these drugs in medicinal doses. The amount of the metal excreted through the urine is comparatively small, and it is questionable whether its detection in this secretion is of practical clinical value except it be studied from a toxicologic standpoint.

Among the alkaloids, quinin and strychnin are often excreted through the urine unaltered, yet here, too, the amount is extremely small. Morphin may be excreted through the urine, but this is not a constant finding following the administration of this drug. It is scarcely within the scope of this volume to deal further with the question of drugs, and the reader is therefore referred to special works upon toxicology.

MICROSCOPIC STUDY OF THE URINE.

Normal urine is acid, and when allowed to stand for a few hours deposits a cloud of variable density at or near the bottom of the vessel, which deposit is sometimes prevented from forming by the rapid development of fungi. This sediment is composed of crystals, epithelial cells, mucus, white blood-cells, and granular débris. Urine voided in the morning is apt to deposit urates, and may at times show uric acid and calcium-oxalate crystals.

Pathologic urines may be alkaline or acid, and are often cloudy when voided, or deposit a heavy sediment on standing; yet it does not necessarily follow that all morbid urines display a precipitate; on the contrary, the urine of chronic interstitial nephritis is exceptionally free from sediment, as is also diabetic urine.

Microscopic study of the sediment from pathologic urine that has not undergone decomposition outside the body always furnishes valuable clinical knowledge and frequently provides the only reliable information on which to base a diagnosis.

SEDIMENTATION.

Two methods are employed in effecting a precipitate of the organic and inorganic constituents of the urine: 1. Allow the urine to stand in a cool place until sufficient sediment has collected at the bottom of the liquid. 2. By means of the centrifuge (Fig. 75); which instrument should be so constructed as to make it impossible for the tubes containing the urine to come in contact with its handle, a defect in many American patterns.

In using this instrument let steadiness, rather than rate of speed, receive first attention. Such instruments propelled by water and by electricity are in use, and I have found them most satisfactory. Shake the urine well and then pour it into the centrifuge tube to within one-fourth of an inch of the brim; place the tube in its special receptacle on the armature (see *Centrifuge*, page 189) and in the opposite receptacle place a tube filled with urine, or with water to balance the instrument. Centrifugate for from three to five minutes, when a precipitate will have collected at the bottom of the tubes. Remove the tubes from the armature and stand them in an ordinary tumbler.

Caution.—The tubes are marked by a small elastic which surrounds the top of one tube.

MICROSCOPIC TECHNIC.

The precipitate is lifted by means of a pipet and a small drop of it placed on the center of a slide, which is placed on the microscope, and the specimen viewed under a low-power objective (two-thirds) to determine its general character. Certain organic and inorganic elements, as phosphates, urates of ammonia, uric acid, epithelia, and casts, are determined through this objective, and fungi may be recognized with a variable degree of certainty.

Light.—In the study of urine a small amount of light is required, since a high degree of illumination prevents seeing many objects of diagnostic value, high light rendering translucent bodies transparent; thus fatty acids, phosphates, tyrosin, soaps of calcium and magnesium, and other delicate crystals, as well as hyaline casts and epithelia, often escape notice when the light is not properly adjusted. To avoid such an error it is my practice to employ an Abbè condenser and an iris diaphragm in the study of all urines. A second diaphragm above the substage is of little value.

The light may also be advantageously modified by interposing colored plate glass or gelatin paper between the mirror and condenser. A complemental color to the stain gives best results; *e. g.*, for methylene-blue use red or yellow; for safranin employ green.

After a specimen has been studied under a low power the slide is removed from the microscope, placed on the table, and a clean cover-glass allowed to fall gently upon it. It may now be studied through a higher power objective. For general urinary work a one-fifth or a one-sixth objective is of most service.

Focus.—These objectives focus at a point just sufficiently distant from the cover-glass to permit the eye to carry between the tip of the objectives and the cover, the head having been lowered to bring the eye on a level with the microscope's table (page 22).

Permanent Mounts.—It is difficult, if not impossible, to acquire sufficient knowledge of the various crystals and their many forms found in urinary sediments, as well as the rare organic substances encountered, to enable one to make a positive assertion as to the nature of any crystal or cell which he may meet in general urinary microscopy; and to insure against all possible confusion, it is necessary to make permanent mounts of interesting specimens. I have employed this method in teaching clinical microscopy in the Medico-Chirurgical College, and believe it to be ideal in its results.

Washing Sediments.—Sediment collected at the bottom of the tubes by centrifugalization may be washed successfully after the following method: 1. Pour off the clear supernatant urine, and to the sediment add sufficient water to fill the tube; then place the thumb over the mouth of the tube and invert it several times until the sediment is equally disseminated throughout the water. 2. Place the tube (sediment and water) in the centrifuge and again sediment. 3. Decant clear supernatant fluid, continuing as above until the sediment has received three or more washings, when it will be cleansed and ready for mounting.

Note.—Washing in no way alters sediments of delicate structure.

If the sediment is to be preserved in crystalline form, a drop of sediment is placed on the center of a slide and allowed to evaporate to dryness in the open air; a drop of Canada balsam or of the following mounting medium is placed on the center of the specimen by means of a glass rod, and a clean cover-glass allowed to fall gently on the medium, which is spread by its weight. Bubbles of air are in no way detrimental to the preservation of the specimen.

Caution.—It requires a large drop of Canada balsam for mounting urinary sediment.

Casts.—Renal casts are preserved by allowing the urine to evaporate nearly to dryness, a drop of the following medium being added to the center of the specimen:

Liq. acidi arsenosi (U. S. P.)	1 fl oz.
Salicylic acid	½ gr.
Glycerin	2 fl. dm.

Warm slightly until solution is effected, when add acacia (whole tears), and again warm until solution is saturated; after subsidence decant clear supernatant liquid. A drop of formalin, 40 per cent., may be added to this mixture.

It will be noticed that the urine immediately surrounds the drop of medium, and in order to get the casts equally distributed throughout the slide it is necessary to carry a fine needle from the outer margin of the urine (wash-water and sediment) to the center of the medium until the two substances show no tendency to separate. A cover-glass is moistened by the breath and then allowed to fall gently upon the specimen. The slide is now put in a cool place for from twenty-four to forty-eight hours (see *Casts*, page 283), when it should be provided with a permanent ring of microscopic cement.*

URINARY SEDIMENTS.

It has been deemed advisable to divide all sediments of the urine, whether collected by allowing the urine to stand for several hours in a cool place or by the aid of the centrifuge, into two classes; viz., organic and inorganic sediments.

The accompanying scheme will be found serviceable as a guide to the scientific research necessary for the determination of urinary sediments, and will refer the student to a more elaborate discourse upon the substance in question.

INORGANIC SEDIMENTS.

These may be either crystalline or amorphous in character, and their physical properties will be governed largely by the chemic reaction of the urine in which they are found. The reaction—time since voided—and the naked-eye appearance of the sediment aid materially in making a positive determination, since one encounters crystals similar in microscopic appearance though widely different in composition.

For convenience of study inorganic sediments are separated into two classes, viz., those found in acid and those occurring in alkaline urines.

Acid urine, which on standing displays a heavy sediment of a reddish color, is suggestive of urates, and if entirely due to this substance, the turbidity disappears on heating (see *Scheme*, page 261). When large crystals of uric acid are also present, we may observe fine, reddish-brown, sand-like particles at the bottom of the urine, and when the bottle is inclined so as to displace the urine, these sands may cling to its sides (see *Uric Acid*). A scarcely perceptible, flocculent precipitate seen near the bottom of the bottle suggests oxalates. Certain pigments from the blood and bile may at times discolor the urine and its sediments.

Alkaline urine on standing is apt to display a more or less heavy sediment. When such sediment is easily broken by shaking, phosphates are probably present; but when it is seen to be ropy and is not readily lifted into the pipet, pus and mucus may also be present. Heat increases the precipitate in either case, but turbidity due to phosphates disappears on the addition of acetic acid. Indican may be present in connection with a heavy sediment and lend its color to the precipitate.

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* "N. Y. Med. Jour.," Nov. 4, 1899.

I. Transfer some of the Urinary Sediment to a Test-tube by means of a Pipet, and to it add some of the Supernatant Urine. Shake Well.	II. Physical Properties of the Sediment.	III. Tests to be Applied to the Sediment Deposited at the Bottom of the Urine, Spontaneously or by Centrifugalization; and to that Separated by Filtration.	IV. Reaction of the Urine.
1. Fill the test-tube to one-half its depth with turbid urine and heat the upper portion gradually to the boiling-point. Should the turbidity disappear, the substance is urates (page 274).	1. May be brick-red, grayish red, or even milky; and when agitated, the entire urine assumes this color.	1. Murexid test (page 196). Moisten with strong nitric acid, evaporate to dryness. Avoid a temperature higher than 110° C. The yellowish-brown mass which remains changes to purple after the addition of a drop of ammonia water, and to violet when potassium hydroxid is added. (Characteristic of urates and uric acid.)	1. Acid the rule, rarely neutral.
2. Leucin and tyrosin.	2. When present in large amounts gives a yellowish color to the urine.	2. Examine for microscopic crystals of leucin and tyrosin (see page 267).	2. Acid, rarely alkaline.
3. Sediment almost insoluble, coarsely granular, and of a brick-red color—uric acid.	3. Collects at the bottom of the urine and upon the sides of the vessel, giving it a peppered appearance.	3. Murexid test (page 196).	3. Acid.
4. Should the turbidity increase upon agitation and heat, the substance is probably one of the following: Calcium carbonate; or	4. White flocculent or dense precipitate which causes effervescence upon agitation.	4. Soluble in acetic acid with decided effervescence (page 277).	4. Alkaline.
5. Earthy phosphates; or	5. White or bluish-white precipitate which appears ropy and mucoid upon agitation and at times forms a rather tough mass at the bottom of the urine.	5. Soluble in acetic acid without effervescence (page 277).	5. At times acid, commonly alkaline.
6. Albumin.	6. White precipitate which becomes flocculent when agitated. May or may not appear organized.	6. Is not soluble in acetic acid; gives xanthoproteic test; yellow with strong HNO_3, but changes to orange when NH_3 is added in excess.	6. Acid or alkaline.

I. TRANSFER SOME OF THE URINARY SEDIMENT TO A TEST-TUBE BY MEANS OF A PIPET, AND TO IT ADD SOME OF THE SUPERNATANT URINE. SHAKE WELL.	II. PHYSICAL PROPERTIES OF THE SEDIMENT.	III. TESTS TO BE APPLIED TO THE SEDIMENT DEPOSITED AT THE BOTTOM OF THE URINE, SPONTANEOUSLY OR BY CENTRIFUGALIZATION; AND TO THAT SEPARATED BY FILTRATION.	IV. REACTION OF THE URINE.
7. Where the turbidity increases or remains stationary after the addition of acetic acid, one of the following substances may be present: Mucus (viscid); or	7. Sediment loose and flocculent and never collects to form a compact mass at the bottom of the urine.	7. Dissolves in part or entirely by potassium hydroxid.	7. Alkaline, rarely acid.
8. Pus (sifts into pipet with difficulty, viscid and ropy); or	8. White or greenish-yellow sediment which becomes stringy or ropy upon shaking.	8. Treated with KOH forms a stringy gelatinous mass. Pus-cells may be detected microscopically (page 278).	8. Alkaline or acid.
9. Blood.	9. Both sediment and supernatant urine are reddish or brownish in color.	9. Spectroscopic examination of the sediment reveals the absorption-bands characteristic of blood-pigment (page 222). Hemin crystals may be detected microscopically (page 31); also red blood-corpuscles.	9. Acid or alkaline.
10. Hematoidin and less often bilirubin.	10. Reddish or brownish sediment.	10. Sediment may show bilirubin as well as hematoidin crystals (page 270). Bilirubin when evaporated in a dish and treated with fuming nitric acid becomes green and microscopically displays brick-red rhombohedral plates.	10. Acid or alkaline.
11. Calcium oxalate.	11. A white cloud often situated just above the bottom of the urine, but when large quantities are present, it may form at the bottom of the fluid.	11. Soluble in HCl and may be reprecipitated by the addition of ammonia water. Insoluble in acetic acid. Characteristic crystals microscopically (page 265).	11. Acid.
12. Fibrin.	12. A colorless tenacious sediment or coagulum.	12. Freely soluble in a warm 1 per cent. solution of Na_2CO_3. Gives Millon's reaction (pages 217, 224).	12. Acid or alkaline.

13. Cystin, $(C_3H_6NSO_2)_2$.	13. Colorless precipitate.	13. Soluble in hydrochloric acid but not readily reprecipitated by ammonia water. Characteristic crystals microscopically (page 271).	13. Acid.
14. Cholesterin, $(C_{26}H_{44}O + H_2O)$.	14. Silver-colored scales or flake-like plates collected at the bottom of the liquid, but on shaking are to be seen disseminated throughout the urine.	14. Is insoluble in dilute hydrochloric acid or dilute ammonia water. Dissolves in chloroform. When dissolved in chloroform and added to an equal part of sulphuric acid (specific gravity 1.75), the chloroform layer of the mixture first becomes a yellowish brown, then blood-red, and if allowed to stand exposed to the air, this color changes gradually to blue, then green, and finally yellow. Microscopically the characteristic crystals are found (page 272, also Fig. 92).	14. Acid or alkaline.
15. Bence-Jones' albumose.	15. A rather loose whitish sediment. (Rare.)	15. Gives the biuret reaction (page 217) Millon's reaction (page 217), and the author's sulphur reaction (page 187).	15. Acid, rarely alkaline.
16. Indigo.	16. A blue precipitate.	16. Characteristic bluish crystals microscopically (page 277).	16. Alkaline or acid.

Uric Acid.—Crystals of uric acid are originally colorless, but when present in urine they are colored yellow, brown, or red by urinary pigments, and specimens are seen extending through the successive variations of these shades. Rarely indeed are these crystals devoid of color in this situation. Crystals of uric acid may present many forms, and it is not uncommon to find two or more varieties represented in the same specimen, which necessitates that the student study many urines containing uric acid in order to become familiar with its many characteristic crystalline forms. The accompanying plate (Plate 15) and illustration (Fig. 94) were sketched after a careful study of several slides—permanent mounts —collected from the author's clinical studies; yet they fail to show many equally characteristic though not common crystalline forms.

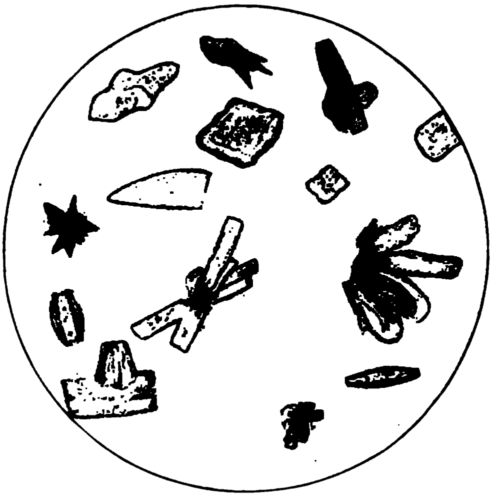
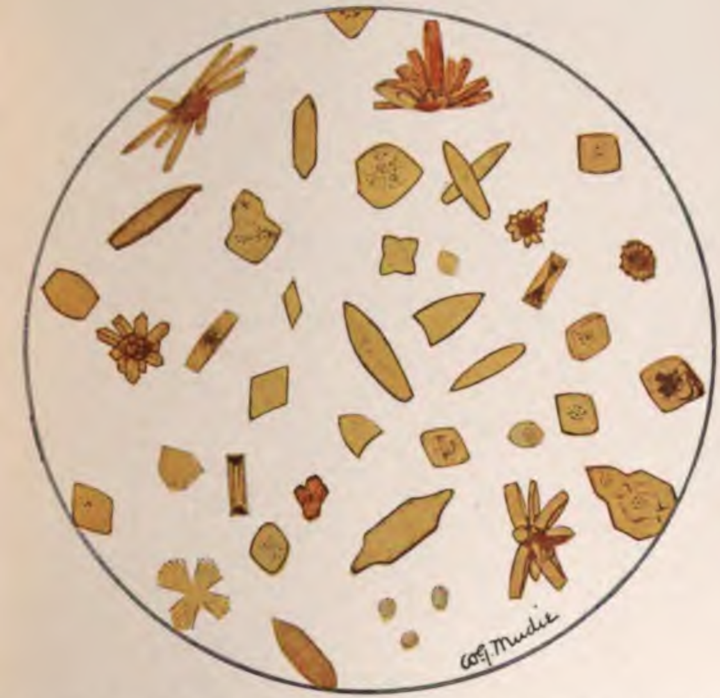


Fig. 94.--Uric acid. From selected slides (obj. Queen one-sixth).

Crystals.—The size of these crystals varies greatly even in the same form. Among the commoner forms are: The irregular diamond shape (flat-iron) crystal, which is often poor in coloring-matter and may present a central spot resembling a nucleus or the outline of a smaller central diamond; rhombic plates with more or less rounded ends richer in color, thicker, more highly refractive, showing a distinct central spot and at times longitudinal striations (whetstone crystals); spheric or oval crystals from the surface of which project one or more spines; long forms presenting a rounded surface with a central diameter slightly greater than that of either extremity (cigar crystals); short, thick forms with

PLATE 15.



Crystals of Uric Acid from Permanently Mounted Specimen Slides
(obj. B. and L. one-sixth; eye-piece 2).

Philadelphia Hospital, with the following diagnosis: acute mania, hepatic abscess, and cocain poisoning. Dr. George Pfahler (through whose courtesy my studies were conducted) has made a detailed report of his findings in these cases.*

Crystals.—These crystals appear in needle-like forms, and when studied singly under a moderately high-power objective (one-eighth), are seen to taper decidedly at each extremity, while at the center two slight lateral depressions are observed (Fig. 97), which apparently favor their peculiar arrangement. By careful focus and a feeble light the clusters (rosets) are found to be formed from a crossing of several of these needles, which focus at different levels so that when one layer is in focus the others appear indistinct and slightly shortened, giving somewhat the appearance of a chrysanthemum. Similar crystals are found in feces.

These crystals do not respond to the tests for tyrosin, and on careful study are seen to differ widely from the crystals of that body, though imperfectly formed sheaths are occasionally seen.

Neutral Calcium Phosphates.—This substance in the urine may appear as delicate, colorless prisms, and less commonly is it seen to take the form of fine needles, which may collect either in bundles or rosets (Fig. 98), in which instance it resembles tyrosin. Such crystals occur in neutral or faintly acid urines, and are to be detected when such urines are changing from acid to alkaline.

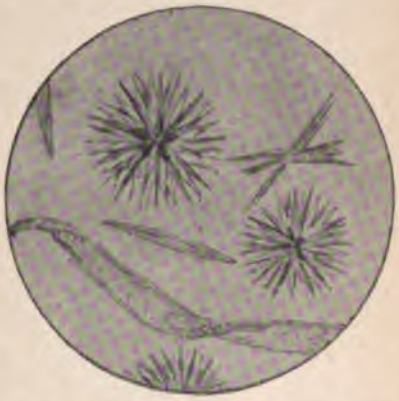


Fig. 97.—Crystals of soaps of calcium and magnesium. Shred of mucus in lower portion of field (from Pfahler's case) (obj. B. and L. one-sixth).

Solubility.—They are dissolved by acetic acid and by ammonia.

Boric Acid.—After toxic doses of boric acid it may appear in the urine as an amorphous sediment. I am indebted to Dr. John A. McKenna for a specimen of such urine that was acid in reaction, cloudy, of high color, and showed a heavy sediment, which was composed for the most part of amorphous boric acid.

Tyrosin and Leucin.—These substances when present in the urine in considerable amounts appear in the following crystalline forms:

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* "Phila. Hosp. Rep.," 1900.

Tyrosin assumes the form of fine colorless needles of equal diameter throughout their length. They are commonly seen in bundles showing marked constriction at the center (sheaves).

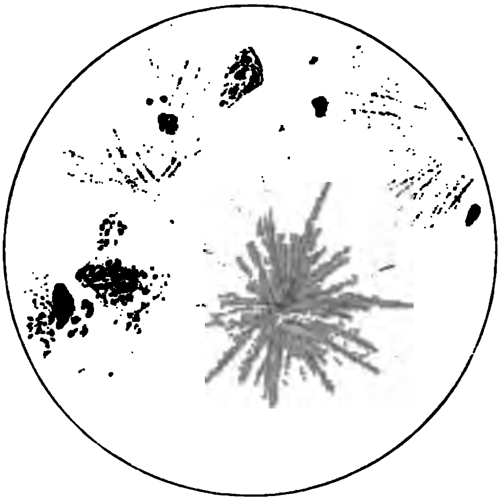

Fig. 98.—Neutral calcium phosphate (obj. B. and L. one-sixth).

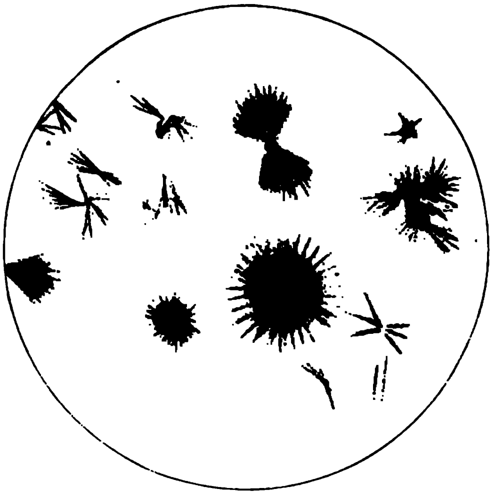

Fig. 99.—Crystals of tyrosin from case of jaundice with enlargement of liver observed at Philadelphia Hospital (obj. B. and L. one-sixth).

A sheaf may be broken at its center, and each half resemble an open fan (Fig. 99). These needles, when in alkaline medium, occasionally unite to form rosets, resembling somewhat the soaps of calcium and magnesium, and of neutral calcium phosphates, yet to the careful observer decided differences are apparent.

Solubility.—By adding a drop of either an acid or an alkali to the slide, the crystals are seen to dissolve as they come in contact with these substances. Dissolution is also effected by heating the slide, when an odor of phenol is emitted.

Clinical Significance.—Tyrosin being a product of the decomposition of proteids, appears in the urine in large amounts only when such changes are rapid, and is found in connection with leucin during the course of acute hepatic atrophy, acute phosphorus-poisoning, and rarely in sepsis, typhoid fever, leukocythemia, small-pox, and other infectious maladies.

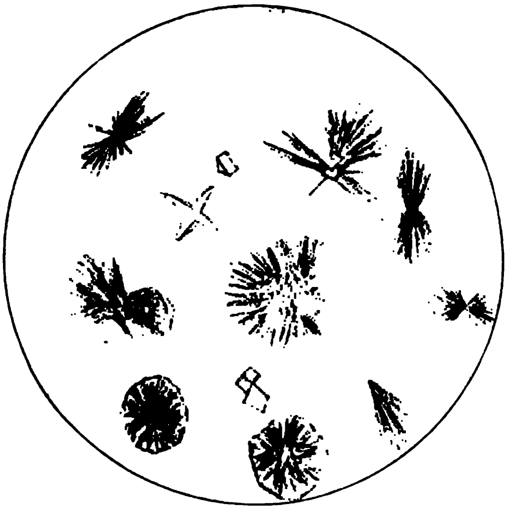

Fig. 100.—Leucin discs and tyrosin crystals (obj. Spencer one-sixth).

Leucin.—Leucin is often found in the urine showing tyrosin, and is the result of similar retrograde metabolic changes. Like tyrosin, but a small amount of this substance is crystallized to form a sediment, and it may be present in a comparatively large amount and yet give no microscopic evidence whatever.

Leucin, when crystalline, occurs as small, highly refracting, yellowish spheres, slightly resembling oil-globules (see Figs. 100, 101). Aggregations of these crystals in conjunction with amorphous material are common findings, and when pure, the crystals appear as extremely delicate plates; roset crystals are rarely seen in fresh urines, but standing encourages their development.

Solubility.—Crystals of leucin are not changed by ether, which distinguishes them from the fat-globules. They are dissolved by caustic alkalis.

Bilirubin.—Urine containing large amounts of bilirubin may present this substance in crystalline form, which is seen either as rhombic plates or as fine needles and which may be distributed singly or in clusters varying in color from yellow to dark red.

If a drop of nitric acid be added to the margin of the cover-glass and the specimen carefully watched while the acid enters beneath the glass and mingles through the urine, each crystal will appear to be surrounded by a greenish band. Crystals of bilirubin are dissolved by caustic soda.

Clinical Significance.—The urine from severe forms of jaundice may contain crystals of bilirubin which should always be distinguished from tyrosin—a common finding in this situation.

Hematoidin.—Unlike bilirubin, hematoidin assumes many crystalline forms, rhombic or oblong plates, ill-formed masses, small discs from which fine needles are seen to radiate, and occasionally a crystal resembling the limb of a tree, displaying fine branches on one or both sides (Plate 17). Their color is red and varies according to the shade of the urine in which they are suspended.

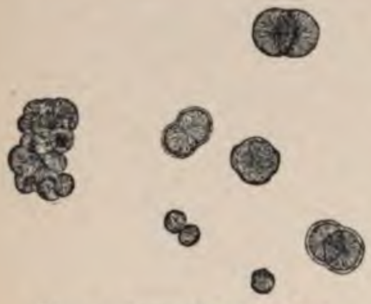

Fig. 101.—Leucin crystals (Ogden).

Distinction.—When treated with impure nitric acid, a play of colors is observed changing to a transitory blue. Bilirubin when thus treated changes to green. Bile-pigments, too, rarely display a blue color, which therefore renders the nitric-acid test of little use with biliary urines.

Solubility.—Crystals of hematoidin are insoluble in acetic acid and partially soluble in ether.

Clinical Significance.—Hematoidin may be found in the urine during the course of acute infectious fevers, acute yellow atrophy (hepatic), phosphorus-poisoning, jaundice, hepatic cirrhosis, or carcinoma, and following the rupture of vessels or abscesses into the urinary tract; and after renal trauma.*

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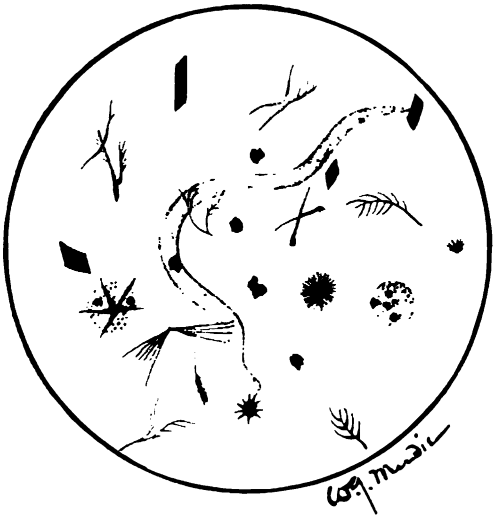
* Yarrow, "N. Y. Med. Jour.," Jan. 6, 1900

PLATE 17.



Hematoidin Crystals and Filaria Sanguinis Hominis from the Urine.

Through the courtesy of Dr. John A. McKenna I was provided with a number of specimens of urine from a patient from whom one kidney had been removed and which was found by the surgeon to consist, for the most part, of a cyst or of an abscess (?) containing milky fluid. The urine in addition to showing many crystals of hematoidin also contained a few small parasites (not determined) (Plate 17) which were lifeless.

Triple Phosphates.—Crystalline ammoniomagnesium phosphates are to be found in feebly acid specimens (see *Alkaline Sediments*, page 275).

Solubility.—Crystals of phosphates are readily soluble in acids.

Basic Phosphates of Magnesium.—Urines found to be faintly acid, alkaline, or neutral in reaction at times contain this substance in crystalline form, when it appears as highly refracting, elongated rhombic plates resembling somewhat the envelops of calcium oxalate, but they are more massive and less perfect. Again they are seen to resemble a clover-leaf in form.

Solubility.—They are dissolved by acetic acid and may be reprecipitated on the addition of sodium carbonate. No special clinical value is attached to their presence.

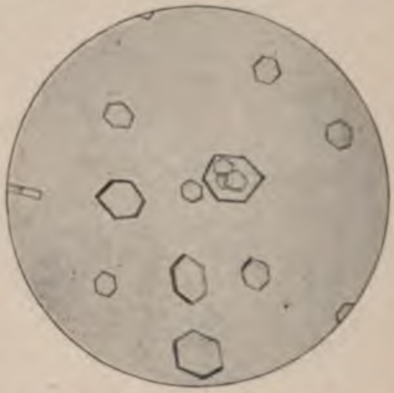


Fig. 102.—Crystals of cystin. Study through the courtesy of Dr. J. M. Anders (obj. B. and L. one-sixth).

Hippuric Acid.—This substance is rarely present in human urine, and is said to follow the ingestion of cranberries or the administration of benzoic acid. Its crystals are colorless, four-sided prisms, soluble in both hydrochloric acid and ammonia.

Cystin.—Urine containing cystin is not apt to be decided in reaction, and if a considerable amount be present, it is likely, on standing, to emit an odor of sulphureted hydrogen. It does nor follow, however, that such urine will always give microscopic evidence of this substance in its precipitate. The addition of acetic acid causes cystin to be thrown out of solution, and appear in the form of colorless, hexagonal plates with clearly defined margins (Fig. 102).

Solubility.—They are soluble in ammonia and hydrochloric acid, but unaffected by alcohol, ether, acetic acid, and water.

Clinical Significance.—The urine contains cystin in the presence of vesical calculi formed from this substance, and cystinuria may persist after the removal of such calculus.

Xanthin.—This substance must indeed be rare, Purdy estimating the total number of cases reported at less than one dozen. Morphologically, crystals of xanthin resemble those of uric acid, but are colorless. A noteworthy fact, however, is that colorless crystals of uric acid may be seen. Chemic analysis is therefore necessary to decide between these two substances.

Cholesterin.—Urine containing this substance is feebly acid, as a rule, and of normal color or slightly turbid. On standing, the precipitate contains the characteristic crystals (Fig. 92), and when it is disturbed by shaking, fine, scale-like flakes are seen to float in the liquid. Under the microscope these crystals appear as thin, translucent, glassy plates, often overlapping one another.

Clinical Significance.—Urine containing chyle, and where certain cysts—hydatid or others—have communicated with the genito-urinary tract, may show cholesterin.

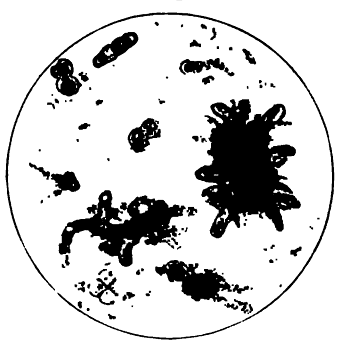
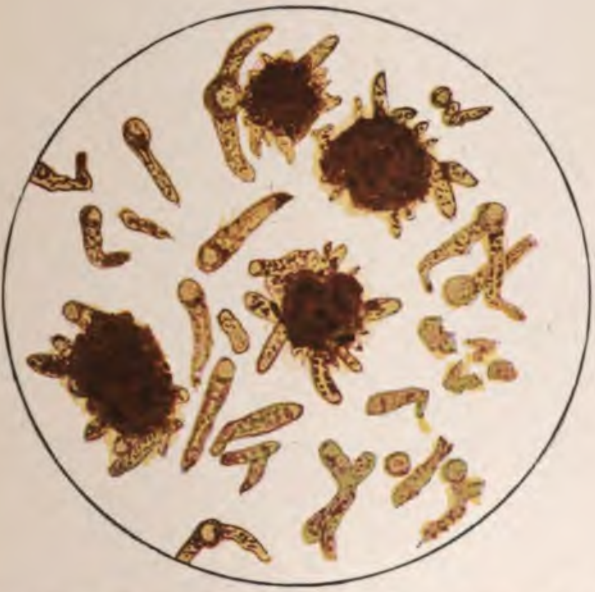


Fig. 103.—Acid ammonium urates from case of rheumatism (obj. Queen one-sixth).

Acid Ammonium Urates.—Occasionally we find crystals of ammonium urates in acid urine, but in such instances the urine contains an unusually large amount of amorphous urates or other sediments common to acid urine, as oxalates. When urine is but feebly acid, and during the change from acid to alkali,—the result of standing,—typical spheres and chestnut-burr (thorn-apple) crystals may be seen. In fresh acid urine, urate of ammonia appears in the form of spheres from which project one or more spines, often parallel to one another, with sharp or blunt points, and appear to be formed from a coarsely granular substance (Fig. 103 and Plate 18). Many of these crystals unite to form huge, dark-brown or yellowish masses, showing blunt, spine-like projections which are seen at different points of focus. Chains formed of from three to five perfect spheres, having the appearance of a segmented block with rounded ends,—by far the most common forms of crystals of acid urates,—are often present without the above forms. Again, there are bottle-shaped crystals which may be clustered (Plate 18), and less often rosets composed of four or more spheres are seen; but true dumb-bells are rare. All the above forms are more or less deeply colored, varying from a

PLATE 18.



CID AMMONIUM URATE (from a case of chronic rheumatism studied in the Pennsylvania Hospital).

light yellow, in the smaller varieties, to yellowish brown in the larger forms.

Clinical Significance.—Rieder reports having studied these crystals from a single case. I have observed them in fresh acid urines on four occasions: One being found in a case of rheumatism treated at the Pennsylvania Hospital; another, with a diagnosis of gout, was studied through the courtesy of Dr. Henry Cattell; and the remaining cases gave histories of rheumatism and were observed at the Philadelphia Hospital, one suffering from acute alcoholic delirium, the other from acute mania. The segmented block and the bottle-crystals I have seen in the urine of tonsillitis and of acute articular rheumatism.

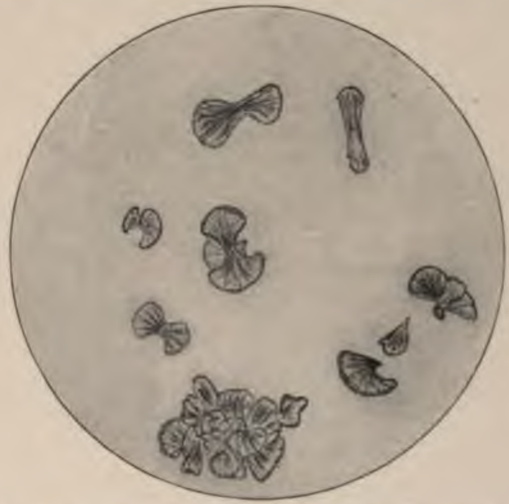


Fig. 104.—Sodium-urate crystals from urine of a gouty patient observed at Pennsylvania Hospital (obj. Spencer one-sixth).

Acid Urate of Sodium.—The greater portion of this substance appears in the urine as granular débris, but crystals may also be seen (Fig. 104) in the form of fine needles (Fig. 105). These needles may unite to form imperfect sheaves, which, when broken into halves, have the appearance of an opened fan. They are originally colorless, but may be colored by the urinary pigments, and are therefore the same shade as the urine from which they are precipitated. Acid urate of sodium enters into the formation of sediments containing uric acid.

Solubility.—It is soluble in 124 parts of boiling water and 1150 parts of cold water (Purdy).

Amorphous Deposits.—Urates.—This variety of urates is a common cause of the highly colored turbidity of urine from plethoric and febrile conditions (tonsillitis); but when gentle heat is applied such turbidity disappears. Microscopically, this substance appears as small granules arranged in masses, which under a one-sixth objective present a slight yellowish tinge. Such sediment when treated with acid may precipitate rhombic plates of uric acid.

Calcium Sulphate and Oxalate.—Both of these substances may appear as irregular dumb-bells accompanied by a variable amount of amorphous material. Sulphates are insoluble in concentrated hydrochloric acid, which dissolves the oxalates. Oxalates are insoluble in acetic acid.

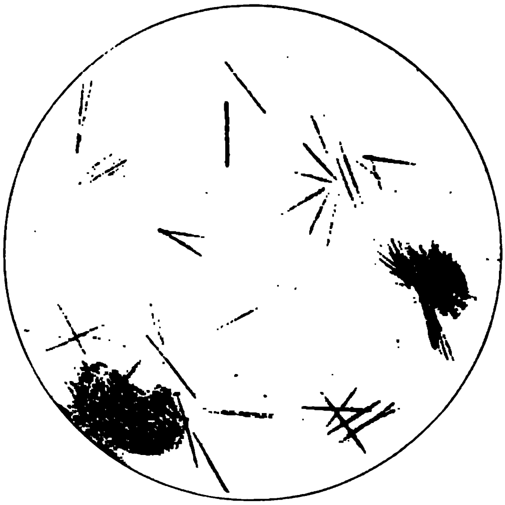


Fig. 105.—Crystals of a complex urate (?) which crystallized on cut surface of a gouty node that had been removed from a toe; observed at Pennsylvania Hospital (obj. Queen one-sixth).

Fat.—Highly refractile bodies of varying size are always suggestive of oil-globules. They are soluble in ether, and stain pink or red when treated with Sudan III, or black with osmic acid.

Clinical Significance.—Fat may appear in the urines of parasitic chyluria, phosphorus-poisoning, and chronic Bright's disease with fatty degeneration of the heart.

Dark Bodies.—Many dark amorphous bodies are seen in the urines of jaundice, hematuria, and fevers. Such findings have been described under their respective headings, and one must rely on chemistry to determine their true composition.

Acid Calcium Urate.—This is a rare urinary deposit and appears as a whitish or grayish powder, which is dissolved with difficulty; and from which, when fused, a white residue of calcium carbonate remains.

Acid Potassium Urate.—This substance forms part of the amorphous deposit seen in acid urine. It is more soluble than is acid calcium urate.

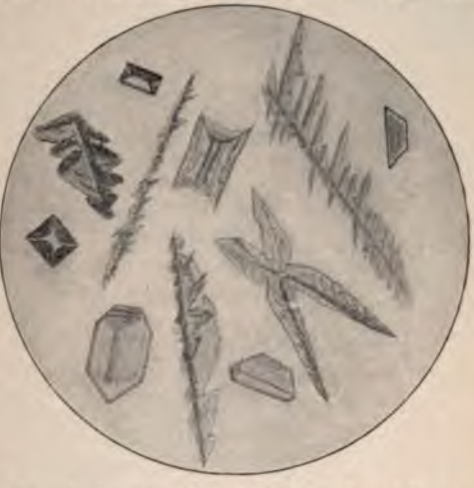


Fig. 106.—Crystals of phosphates (obj. B. and L. one-sixth).

Crystalline Sediments from Alkaline Urine.—**Triple Phosphates.**—This substance (crystalline ammoniomagnesium and calcium) when crystallized from urine may assume many forms, which vary with the degree of alkalinity and whether or not such alkalinity is the result of ammoniacal fermentation. Most constant are the coffin-lid and building-stone crystals (Fig. 106); yet many other forms are seen, among them being the clothes-pin crystal (Fig. 106) and the feather crystal (Fig. 106), which at times resembles the fin of a fish, and which, when so broken as to permit a view of the ends of the fin's ribs, resembles a number of rounded timbers standing on their ends, for which reason I have presented it to my students as the "pile crystal"—resembling the group of piles

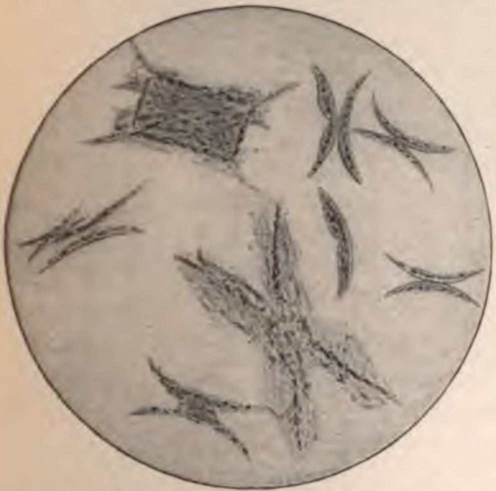


Fig. 107.—Crystals of phosphates from a case of phosphaturia (obj. Queen one-sixth).

which project from the end of a wharf. A crystal bearing likeness to the reel used by fishermen is occasionally seen; as are also heavy, step-like blocks of varying sizes (Fig. 106). Square, oblong, irregularly jagged, snow-flake, and leaf-like forms are also among the findings.

Solubility.—Acids are usually destructive to triple phosphate crystals from alkaline urines.

Clinical Significance.—Flatulent dyspepsia may cause a deposit of calcium phosphate from mixed alkali. Constipation and certain nervous affections, phosphatic vesical calculi, and cystitis contribute toward phosphaturia. It is not possible to estimate the amount of phosphates from microscopic study alone (see page 181).

Urate of Ammonium.—This substance is commonly crystallized from urines of high specific gravity, displaying a sediment containing calcium phosphate and triple phosphates in addition; while urines of lower density are apt to precipitate their urates in the form of uric acid; however, ammonium-urate crystals may be present in urine of low specific gravity.

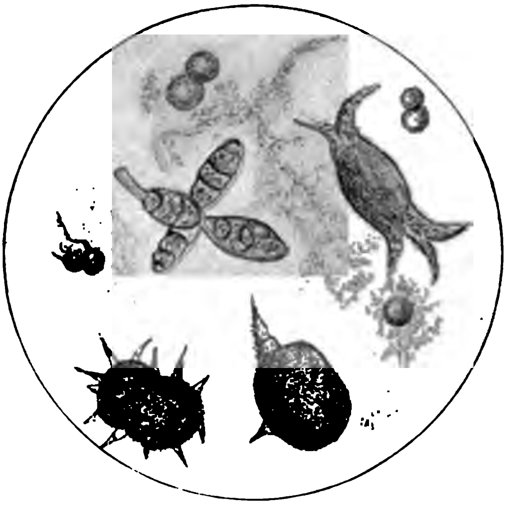


Fig. 108.—Ammonium urates, showing segmented bottle-crystals and thorn-apple formation.

The most constant crystal of this substance is a small, yellowish brown sphere. These spheres are seen singly or in groups of four to six, when they form a crude roset. Smaller spheres occur in pairs connected by an isthmus (dumb-bell); large spheres showing one or more small spines projecting from their surfaces (chestnut-burr or thorn-apple) are also seen. These spicules may be straight or slightly curved (Fig. 108), and rarely are they seen apart from spheres. Crystals of ammonium urate are distinguishable from those of uric acid, acid urate of ammonium, and sodium urate by the fact that they are the only urate crystals found in alkaline urine.

Solubility.—They are dissolved by phosphoric and acetic acids, after which they may precipitate as rhombic plates of uric acid.

Indigo.—It is common for indigo to appear in the urine as amorphous débris, and needle-like crystals showing a bluish tint are occasionally seen. They are formed probably from indoxyl sulphate during the process of decomposition. Crystals of indigo may be found in either acid or alkaline urines, and in form varying from that of needles to oblongs and squares; their shape being modified by the crystals or débris upon which they are collected.

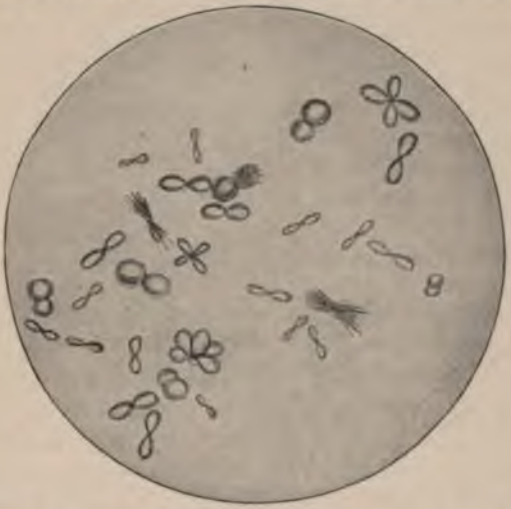


Fig. 109.—Crystals of calcium carbonate (obj. Spencer one-sixth).

Clinical Significance.—Indigo when found in the urine is a symptom of cholera, empyema, neoplasm, conditions favoring intestinal putrefaction, constipation, peritonitis, tabes dorsalis, jaundice, and general cachexia.

Amorphous Alkaline Deposits.—**Carbonate of lime** occurs in the urine in coarse granules and as colorless dumb-bells (Fig. 109) and evolves gas when dissolved in acetic acid. Carbonates of alkaline earths are dissolved without the evolution of gas.

Basic phosphatic earths occur as large and small granular bodies, which, when treated with acetic acid, generate gas during their dissolution.

Urinary concretions are often apparent to the naked eye, and may consist of uric acid, phosphates, cystin, oxalates, xanthin, carbonates, and cholesterin. These will be discussed under their respective heads.

ORGANIC SEDIMENTS.

Leukocytes.—A few isolated leukocytes are present in normal urine, but when present in large numbers they are of pathologic origin. They are often increased after the worship of Venus and Bacchus, though in such urines they are well preserved and present clear nuclei and their usual smooth and glossy appearance. Leukocytes when present in alkaline urine soon become lusterless, swollen, and their nuclei become indistinct. By placing a drop of acetic acid at the margin of the cover-glass and watching the specimen closely through the microscope, it will be seen that all cells coming in contact with the acid at once display more clearly their nuclei. Add a drop of iodopotassic-iodid solution in place of the acid, and the leukocytes stain a faint mahogany shade which serves to distinguish them from epithelia, which stain yellow. Pus-cells, when treated with liquor potassa, show a tendency to swell and coalesce, their nuclei being seen with difficulty.

Clinical Significance.—The leukocytes of the urine may be derived from any portion of the urinary tract through congestion, or from abscesses of adjacent structures rupturing into the urinary channel. It is the rule that when a limited number of pus-cells are found equally distributed through the urine, they are of renal origin (pelvic); while a large or small amount of pus, not thus disseminated throughout the urine and found in connection with large epithelial cells, may be regarded as being of cystic origin.

Pus.—The pus of purulent catarrh of the bladder and of acute urethritis (gonorrhea) forms a heavy viscid sediment, and the cellular elements are commonly much distorted.

Pus-cells occur in the urine in case of inflammation of the ureters and in pyelitis, but here the sediment is less marked (Fig. 110) and often has a light, flocculent appearance, while that from the bladder is ropy and not readily broken into flocculi by shaking. Pus from abscesses may resemble that of cystitis except for the particles of fatty matter seen in occasional cells. It is to be remembered that in renal disease often but few leukocytes are to be observed, and that in women they may be of vaginal origin. To determine the source of the pus in the urine is of the greatest clinical value, and may at times be done with a certain degree of accuracy, the exceptions being no less numerous than the rule. Catheterization of the ureters has been proved of service in my experience.

Epithelia.—The deposit which forms in normal urine contains a few epithelial cells, most of which are of the squamous variety, yet smaller forms may be present, since desquamated epithelium is a product of all mucous surfaces.

Clinical Significance.—Both polygonal and round uninuclear cells are derived from the prepuce, meatus, and vagina; while epithelial cells from the bladder are usually flat, irregularly round or angular, the nucleus located nearly central, and is seen without staining (Fig. 111). The epithelial cells from the ureters and renal pelvis are, as a rule, of medium size and often display a caudal projection (Fig. 111, 2). Marked difference is observed between epithelial cells from the superficial and deeper layers covering any mucous surfaces. Small cells, and also those showing tail-like projections, were formerly regarded as coming from the kidneys or renal pelvis, but careful study has shown that cells identical in every respect may originate from the deeper layers covering the vesical mucosa.

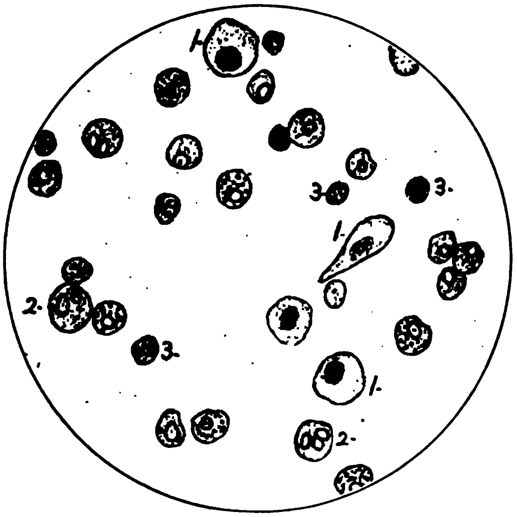


Fig. 110.—Urinary sediment from case of pyelitis: 1, Epithelial cells, probably from pelvis of kidney; 2, large pus-cells; 3, small pus-cells (obj. Spencer one-sixth).

When urinary sediment contains a large number of epithelial cells characteristic of any portion of the urinary tract, it may be inferred that there is an inflammatory process affecting such sections. Where cancer affects these parts, clusters and nests of epithelial cells appear in the urine. Many caudate cells are significant of pyelitis, although such cells may be the product of cystitis. In a sediment containing many small epithelial cells resembling pus-cells (see *Pus*, page 278), where the urine contains albumin, or following infectious fevers (scarlatina), one may safely regard such cells as of renal origin. Casts often contain, and may often be found largely composed of, these cells (see *Epithelial Casts*, page 288).

Disease may alter the general feature of the epithelium, and while epithelium from the kidney is fairly common, the presence of such cells in the urine may not bear a direct relation to the

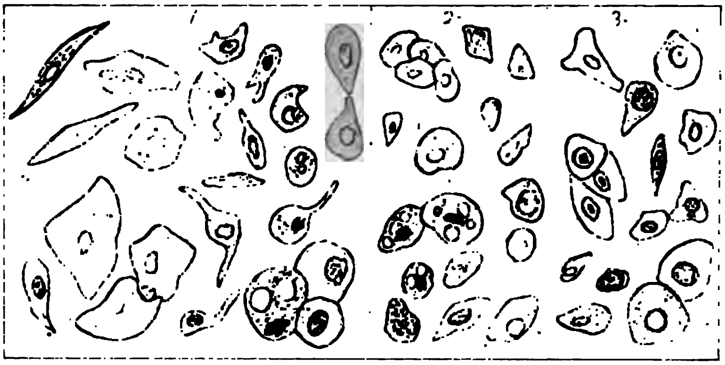


Fig. 111.—1, Epithelial cells from the bladder; 2, from kidney, pelvis of kidney, and from ureter; 3, cells from meatus of male urethra (obj. B. and L. one-sixth).

degree of pathologic condition present. I have been privileged to follow to autopsy many cases of nephritis, and of amyloid kidney, and have found that the number and character of the epithelial cells present in the urine did not bear a direct relation to the lesion present, and at times their number may bear inverse proportion to the apparent damage of the kidney's structure. They were commonly present singly and in clusters (three to six cells each) in cases of toxic nephritis (acute yellow atrophy). Cells showing fatty and amyloid change point with a variable degree of certainty to similar changes in the kidney's structure, yet by no means is this finding constant or even common at postmortem.

Our present knowledge in no way warrants the determination of the origin of any given epithelial cell present in the urine; but in connection with the clinical features of a given case such deductions may be drawn with safety (see *Epithelial Casts*, page 288).

Blood (Hematuria).—Red blood-cells appear in the urine as a result of pathologic changes, either in a portion of the urinary tract or in the blood itself (see *Malaria, Filaria Sanguinis Hominis*, and *Distoma*, pages 153, 147, 147). Blood-corpuscles vary in their general characteristics (see *Erythrocytes*, page 81), depending on the portion of the urinary tract from which they are derived, and the reaction and consistence of the urine in which they are found (see *Osmotic Tension*, page 73). Blood-corpuscles derived from the kidneys rarely increase the specific gravity, are equally distributed throughout the urine, never form rouleaux, and retain their form well if the urine be acid; but in alkaline urine they become swollen, showing slight color, and, should the urine be diluted, appear as mere rings. They present deep color and thickening where the urine is concentrated. Corpuscles derived from the renal pelvis display these peculiarities to a lesser degree.

Clinical Significance.—Blood from any portion of the urinary tract causes a change in both the color and sediment of the urine, bearing a direct ratio to the quantity of this tissue present. It causes a dark-red color in acid urine and a bright-red hue where the urine is alkaline. Blood derived from the renal substance and pelvis lends to the urine a general cloud or tint, while that from the urethra, bladder, and other portions of the urinary tract is not found to be equally diffused throughout the urine, and it forms a heavy sediment, clots readily, or may even be voided in clots.

Hematuria may result in the course of severe anemia (leukemia) or of certain infectious fevers, either as the result of renal congestion or of an altered condition of the blood itself (malarial hematuria). It may develop as the result of acute nephritis, renal abscess, tuberculosis, calculi, pyelitis, cystitis, urethritis, trauma, and a complication in cardiac and hepatic conditions. Tropic hematuria may be produced either by the distoma (page 147) or by the filaria (page 147).

Other animal parasites capable of causing bloody urine are: T. echinococcus, Oxyuris vermicularis, ascarides, Rhabditis genitalis, Trichocephalus, Anguillula aceti, and the ameba (pages 292–300). Infection of the genito-urinary tract by the various vegetable parasites (fungi and bacteria) may often induce hematuria (pages 300–307).

Sloughing mucous surfaces located along the urinary tract, urethral stricture and inflammation are among the rarer causes.

When taken into the system, drugs may induce hematuria. The urine may display peculiarities, such as odor, color, or chemic changes, all of which are equally suggestive of the cause.

DISTINGUISHING FEATURES BETWEEN RED BLOOD-CELLS, LEUKOCYTES, PUS-CELLS, YEAST-CELLS, EPITHELIAL CELLS, AND STARCH.*

ERYTHROCYTES.	LEUKOCYTES.	PUS-CELLS.	YEAST-CELLS.	EPITHELIAL CELLS.	STARCH-GRANULES.
1. Cells of uniform size, pale greenish tinge, and show biconcavity upon careful focus. Central spot circular.	1. Cells larger, less uniformity of size; pale milky color, upon careful focus distinct nuclei.	1. Stroma of cell pale or granular. Outline indistinct. Degeneration of protoplasm and nuclei.	1. Size irregular, color milky blue; cells are elliptic (often). By focus central spot can be made to vary in size and is never round.	1. Protoplasm solid, irregular in size, pale, and shows a pale central nucleus.	1. Pale white, refractile, varying greatly in size, and may show lamination.
2. Cells situated on their edge appear as dumb-bells.	2. Absent.	2. Absent.	2. More elliptic in outline.	2. Absent.	2. Absent.
3. Mycelium absent.	3. Absent.	3. Absent.	3. Mycelia are always to be found, though often few in number (see *Fungi*, page 300).	3. Absent the rule.	3. Absent the rule.
4. Slight effect.	4. Slight staining.	4. Iodopotassic iodid stains cells mahogany brown.	4. No decided staining.	4. Stained pale yellow.	4. Iodin induces a blue color.
5. Not altered by acetic acid.	5. Nuclei rendered rather indistinct.	5. Nuclei made prominent.	5. No effect.	5. No decided change.	5. Negative.
6. Cells present a life-like luster.	6. Physiologic luster present.	6. Dull or lusterless in appearance.	6. Decided luster, and glaring refractive surfaces.	6. Smooth, slightly refractive surface.	6. Highly refractive.

* Consult also figures 93, 110, 111, 115.

CASTS (TUBE-CASTS; RENAL CASTS).

History Note.—The presence of renal casts in the urine was first described by Vigla*; and Henle† called attention to their presence in the urine of persons suffering from dropsy.

Formation.—Different theories have been offered as to the method of their production; but the one generally accepted is that coagulable elements of the blood enter the urinary tubules as a result of pathologic changes (irritation), and there serve as a basement lining of the tubule, forming a favorable lodging-place for all granular material that may enter the tubule either at its union with the Malpighian body or that may exude from the tubule's wall, or from the structural lining of the tubule itself. Thus a cast may displace different cellular elements, suggestive of the changes taking place in the tubule of which it is a mold.

Composition.—The substance forming the basement or primary mold of casts is closely allied to proteids, but its true nature is not known. Knoll, after careful study of the chemistry of casts, concludes that their composition is not identical with any known proteid, though waxy casts at times give reactions known to albuminates. In hyaline casts this substance is freely soluble in dilute mineral acids.

Clinical Significance.—Our present knowledge regarding the clinical significance of urinary casts is such as to render this subject one of great interest. Casts are commonly found in urines that appear normal in other respects and when albumin is not present; but such urines are usually voided after rest or sleep. A specimen collected after exercise, or two hours after a full meal, etc., will, as a rule, show albumin in addition. In all such cases under personal observation, in which a more careful investigation was conducted and specimens collected at different hours of the day, albumin was always detected, though often in small amounts. The more closely we study this class of cases, the more we are inclined to regard them as related, at least, to true nephritis, which, if not present, will develop later. The finding of casts in the urine may be regarded as pathologic and significant of nephritis when the slightest trace of albumin is detected two hours after an evening meal, the day having been spent in taking a reasonable amount of exercise.

Classification.—For clinical study renal casts may be classified as follows: unorganized and organized.

Unorganized casts are formed of crystalline or amorphous substances; and are composed of urates in gouty individuals and in case of renal congestion. Casts of hematoidin crystals have been seen.

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* "L'Espérience," 1837, No. 12.

† "Zeitschr. f. rationelle Med.," 1844, vol. i, pp. 61–68.

Organized casts are composed largely of cellular elements or their products, and may be classified according to their composition, frequency of occurrence, and method of production:

1. Hyaline casts, which appear as thin, pale molds of the renal tubules.

2. Granular casts, by far the most common, are composed largely of degenerated cellular elements and granular débris. Amyloid, fatty, pus, and blood-casts are subdivisions of this variety.

3. Casts consisting largely of red blood-cells, epithelial cells, leukocytes, or bacteria.

4. Composite casts, where the larger portion is hyaline, but shows at certain sections granulations due either to cellular elements or to crystals.

5. False casts (cylindroids), which resemble in certain respects hyaline casts but display distinctive features not known to casts.

The size and form of casts are best shown by the accompanying figures and plate (Figs. 112–120 and Plate 19), which demonstrate their marked variations and general characteristics. This variation is at times so marked as to cause those most skilled in their recognition to question the true nature of many bodies found in urinary sediment. Abnormally large casts often take the form of the tortuous tubules, while on the other hand narrow casts may be perfectly straight and of moderate length. Age has no influence upon the size of casts, as shown by Fig. 115, from the urine of a child (girl) four years old, under the care of Dr. J. Allison Scott, and observed during my service at the Pennsylvania Hospital.

Staining of Casts.—Casts are stained by bile when present in biliary urine. Iodin added to the urine stains the casts feebly but stains the other elements more markedly and thus renders them (casts) conspicuous; and a similar effect results from anilin dyes.

Urate Casts.—This variety of casts (Fig. 112) is commonly met with in general uranalysis. They appear to be composed of amorphous urates. At times they closely resemble organic granular casts, in which case an absence of urates suffices for differentiation. Rarely are organic casts seen in the same urine with weak casts. When urate casts meet they show a tendency to coalesce, forming an unorganized mass—a condition not observed if the casts be composed of organic substances.

Their presence in the urine is probably suggestive of renal congestion. The author has found them common in cases of plethora, rheumatism, and tonsillitis. Two women of middle life, now under observation, develop periodic attacks of tonsillitis, at which time their urines contain many such casts. Between these attacks the urine is normal. Urine containing such casts develops a heavy sediment on standing and may present a cloud even when voided (see *Transparency*, page 171).

Casts composed largely of hematoidin crystals have been observed by various writers.

Hyaline Casts.—There is a diversity of opinion as to the method whereby this primary variety of casts is produced, some regarding their formation as due to a special product secreted by the renal epithelium, while others contend that they are formed from albumin or its derivatives, which escapes into the renal tubules.

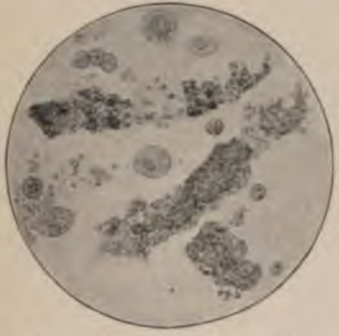

Fig. 112.—Urate casts (pseudo-casts) from case of tonsillitis. Renal epithelia and leukocytes (obj. B. and L. one-sixth).

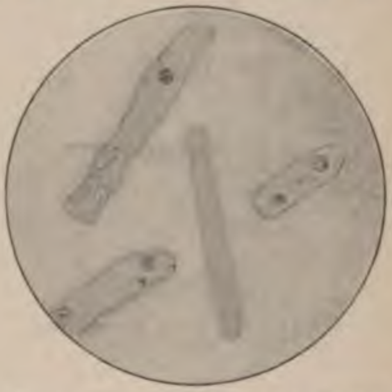

Fig. 113.—Hyaline casts showing fat-droplets and leukocytes (obj. B. and L. one-sixth).

Hyaline casts appear in the urine in variable numbers and are of indefinite proportions. Wide variations exist as regards the diameter of these bodies, and they may vary in length from one-fourth to several times the diameter of a microscopic field; their outline is faint and seen with difficulty, and one extremity occasionally presents a twisted appearance or shows slight tapering. The surface is finely granular and displays not infrequently epithelial cells, leukocytes, or crystals (Fig. 113). Casts of similar composition, but larger, are observed in puerperal eclampsia (Plate 19).

Detection.—Hyaline casts are most difficult of detection and are seen only when the most skilled technic is employed in adjusting the mirror and diaphragm. The interposing of a colored glass between the mirror and the condenser of the microscope is of service. They may appear quite distinct under a low-power objective (two-thirds), but at the same time it is difficult to see them when highly magnified. Staining is often recommended, but in my experience this method of study has proved valueless.

Clinical Significance.—Various writers claim to have found hyaline casts in the urine from perfectly healthy persons, and in cases where postmortem disclosed a healthy condition of the kidneys. I wish to add my experience to that of Leube and Purdy * in that urine containing hyaline casts contains albumin or has recently contained this body, even though in small amounts. Study of a series of similar cases at the Howard and Philadelphia Hospitals resulted in the detection of albumin, at some period, when it was possible to follow the cases and make examinations at intervals of days or even weeks, and of urines collected at different hours of the day, after exercise, a full meal, etc. This variety of cast is most commonly found in chronic nephritis of the interstitial type; it occurs also in other forms of chronic and less often in acute nephritis, catarrhal jaundice, cancer of the liver, stomach, or intestine, and in conditions coupled with anemia. At times casts are stained, due to the presence of bile-pigments in the urine (Plate 19). Hyaline casts are not uncommon during the latter months of gestation and in puerperal eclampsia (Plate 19). The urine may contain hyaline casts for many years (Fig. 114) (ten to thirty) without pronounced albuminuria or appreciable discomfort to the patient.

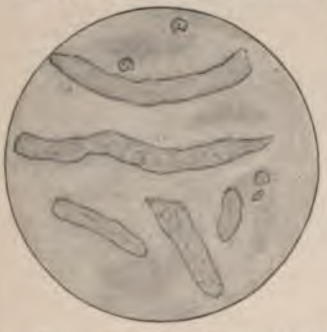

Fig. 114.—Hyaline casts (narrow) from case of contracted kidney. Postmortem, observed at Philadelphia Hospital (obj. B. and L. one-sixth).

Granular Casts.—This variety comprises by far the greater portion of all renal casts, since epithelia, pus, leukocytes, and blood-casts are subdivisions of this class. Granular casts are largely composed of the products of pathologic changes which have become disorganized while in the uriniferous tubules. They are seen to vary greatly in size and general characteristics, depending upon the nature of the existing renal lesion, the time they have remained in the renal tubules, and the degree of acidity of the urine in which they are found, alkaline urine causing them to disintegrate.

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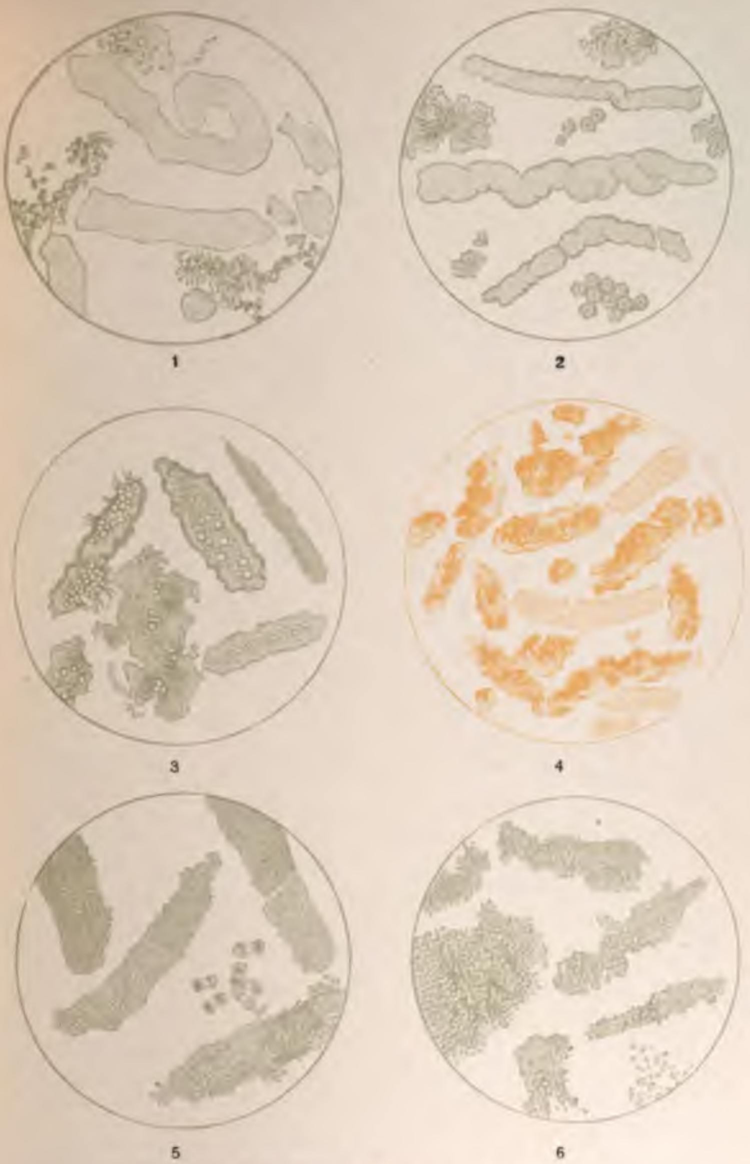
* "Zeit. f. klin. Med.," 1887, vol. xiii, p. 7.



VARIOUS FORMS OF URINARY CASTS.

1. Hyaline casts from case of puerperal eclampsia (original) (obj. B. and L. one-sixth).

2. B. J., age twenty-two, female, suffering from puerperal eclampsia. Urine showing large, finely granular casts (original) (obj. Queen one-sixth; eye-piece 2).

3. S. A., age fifty-eight, male. Urine showing granular and fatty casts; post-mortem showed chronic parenchymatous nephritis (original) (obj. Queen one-sixth; eye-piece 4.)

4. J. D., age fifty-four, male, suffering from cancer of the common duct and head of the pancreas. Urine showed bile-stained casts (original) (obj. Queen one-sixth; eye-piece 4).

5. A. G., age fifteen, male, suffering from acute nephritis. Urine showed granular casts (original) (obj. Queen one-sixth; eye-piece 2).

6. C. A., age nine, male. Scarlatinal nephritis, third week of convalescence. *Urine showed granular casts (original)* (obj. Queen one-sixth; eye-piece 2).

Granular casts present in common certain features (Plate 19): Either one or both extremities is seen to have been broken abruptly or obliquely, often causing a concave or zigzag fracture; or one extremity may taper slightly. Their lateral boundaries appear as slightly irregular, elevated borders which vary according to the variety of granulation presented by each cast. Such casts differ in their constitution: at times their granules are coarse and dark, and again so fine and pale as to be seen only through a one-sixth objective. The latter may show fine crystals, epithelial cells, leukocytes, fat-globules, or crystals of fatty acids on their surfaces (Plate 19). In color, granular casts extend through the successive shades varying from a faint yellow to a reddish brown or a bluish yellow.

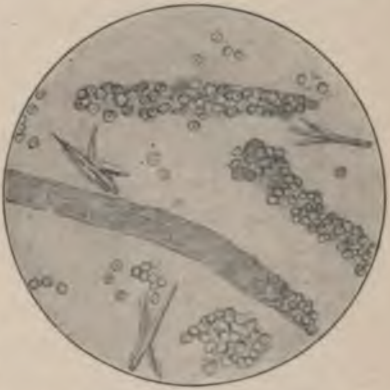


Fig. 115.—Blood-casts and crystals from urine of a child at Pennsylvania Hospital (courtesy of Dr. A. Scott) (obj. Spencer one-sixth).

Clinical Significance.—It is the rule that highly granular casts are short, thick, and commonly found in connection with acute renal inflammation; yet the exceptions are many. Large finely granular casts are observed in puerperal eclampsia and chronic parenchymatous nephritis; while the narrow variety is suggestive of chronic interstitial disease. The variety of granular casts present in the urine offers valuable aid in determining the exact nature of the renal condition present, except where the patient be suffering from an acute exacerbation, relative to some form of chronic nephritis, in which case the casts signify acute nephritis.

Blood-casts.—Generally speaking, blood-casts are short, of uniform diameter, and their margins distinct. When formed of a small quantity of blood-cells, they are light in color and each corpuscle may be outlined by careful focus, but where the renal hemorrhage has been sufficient completely to fill the tubule and to form a mold purely of compressed red cells, they are dark in color and of varying sizes (Fig. 115). Blood-casts are not destroyed in evaporating the sediment of the urine to dryness, and can, therefore, be mounted in Canada balsam.

Caution.—In urine containing a large amount of blood the thickest of its sediment should not be taken for microscopic study, as blood-casts are more easily found in the sediment obtained from the supernatant portion of the fresh urine which renders centrifugation of such urines necessary.

Clinical Significance.—Blood-casts are indicative of renal hemorrhage, but do not imply that permanent structural change has taken place in these organs. They are a rather common feature of the urine of acute traumatic and of toxic nephritis. Figure 125 illustrates blood-casts from a case of hydatid of the kidney studied at the Philadelphia Hospital.*

Epithelial Casts.—The characteristic feature of this variety of casts is that they are formed entirely, or in part, of epithelial cells from the lining surfaces of the renal tubules. They are of moderate size, highly refractive, and often so broken as to allow a portion of an epithelial cell to extend beyond the extremity (Fig. 116); the margins are irregular, and their surface may present a few epithelial cells scattered throughout, or an aggregation of such cells at any point, while the remaining surface appears finely granular *or rarely* hyaline. Casts may be seen when a row of epithelial cells surrounds them in a spiral-like manner, giving the appearance of the thread of a bolt (Fig. 116); again, the free surface may be entirely covered with epithelial cells, which may be granular, swollen, and indistinct, presenting rather poorly defined margins (Figs. 118, 119).

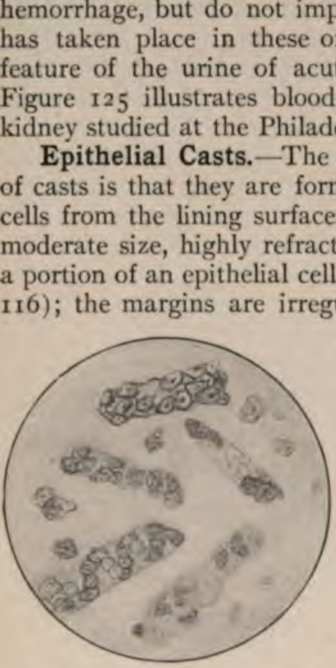


Fig. 116.—Epithelial casts from a case of acute scarlatinal nephritis. Private patient (obj. B. and L. one-sixth).

Clinical Significance. — Epithelial cells containing fat-droplets, and showing marked evidence of degeneration, are to be seen and are fairly suggestive of the presence of a similar condition in the kidney structure.

The finding of epithelial casts in the urine furnishes conclusive evidence of an inflammatory process in the kidneys which is of sufficient severity to destroy the epithelial lining of the urinary tubules. It is a common feature of the exanthemata (scarlet fever), but does not of necessity imply that the kidneys are permanently damaged.

Pus-cells.—It is common to see granular, amyloid, and hyaline casts upon the surface of which a few leukocytes are clinging; and casts composed of pus-corpuscles have been described by Johnson, and observed by the author in a single instance—a case studied through the courtesy of the late Dr. H. E. Gruel

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* "Amer. Med.," Jan. 3, 1903.

(Fig. 117), where the sediment was more flocculent and lighter in color than is that resulting from pus of vesical origin.

Clinical Significance.—Dr. Gruel's patient died of uremia within a few hours after the specimen was collected, and no autopsy was permitted.

Fatty Acids.—Casts showing scattered globules of fat on their surfaces are rather frequent findings, and occur in connection with the amyloid and hyaline varieties found in subacute and chronic nephritis. Aggregations of fatty matter may present needle-like crystals (Plate 19) which doubtless have their origin in the degenerated renal epithelia. Casts composed exclusively of fatty substance are less common and suggest renal disease of a chronic type; yet it does not follow that the kidneys always show a similar change at postmortem. The urine of phthisis and of chronic nephritis not infrequently contains fatty casts, which when composed largely of fatty matter are highly refractive, short, with rounded ends, and rather irregular margins, while their surfaces are studded with oil-droplets. Fatty casts at times contain epithelial cells.

Distinctive Features.—Fatty casts are readily distinguished by allowing a drop of Sudan III (70 per cent. alcohol saturated) or a drop of osmic acid to fall at the margin of the cover-glass, when the cover is so moved as to permit the stain to flow beneath it. The former of these preparations stains pink with fats; the latter, black.

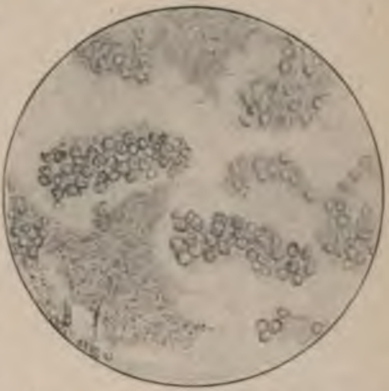

Fig. 117.—Urine containing pus, pus-casts, and epithelia (obj. B. and L. one-sixth). Patient an aged woman.

Amyloid Casts (*Waxy Casts*).—This form of cast is distinguished by its large size and peculiar starch reaction. The casts are refractive bodies, often tape-worm-like in outline, broad, varying from a pale yellow to a yellowish-brown color, and their outline at times raised. Again this outline may be difficult of detection (Fig. 119). Amyloid casts present a greater diameter than do other varieties, and are often of extreme length (Figs. 118, 119); yet specimens may be seen whose length does not exceed the diameter. On their surface are found leukocytes, erythrocytes, epithelial cells, crystals, droplets of oil, and granular débris. They may give amyloid reaction when treated with iodopotassic iodid or with methylene-violet solution, which changes amyloid substances to a blue or a mahogany color respectively; yet this reaction may be absent when the kidneys are amyloid, and present in casts where no such changes are found in the kidneys at postmortem. The exact composition of amyloid casts remains moot.

Clinical Significance.—The presence of hyaline casts in the urine is diagnostic of chronic inflammatory changes in the kidneys, although they may occur in acute nephritis, phthisis, amyloid disease, and in septic processes, of both acute and chronic nature. The darker variety of casts I have found in the urine of puerperal sepsis and of sepsis following operations. The paler forms have been observed (Plate 19) in the urine of puerperal eclampsia.* They are also a feature of chronic nephritis.

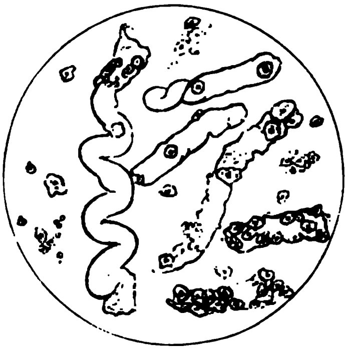

Fig. 118.—Showing epithelial and amyloid casts. Patient a female, aged forty-two years, suffering from septicopyemia with amyloid kidney (obj. Queen one-sixth; eye-piece 2).

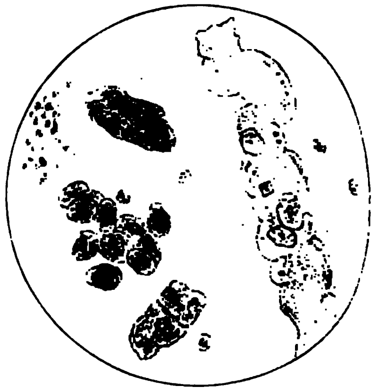

Fig. 119.—Large amyloid cast containing three renal epithelial cells upon its surface. Two short hyaline casts; cluster of renal epithelium; and few leukocytes (obj. B. and L. one-sixth).

Cylindroids.—These bodies were detected in the urine from scarlet-fever patients by Thomas.† They appear as ribbon-like bodies, rather lusterless, and drawn to a fine point at one extremity (Fig. 120). Their surface is commonly free from granular débris, but may show fine lines extending parallel to their lateral borders, as well as transverse elevations giving to the cylindroid the appearance of a twisted tape.

Clinical Significance.—Cylindroids are found in the urine of persons suffering from renal congestion, nephritis, pyelitis, and cystitis. They may be found in urine containing an excess of amorphous urates when the urate granules congregate upon the cylindroids, thus forming the *so-called* "urate casts" (Fig. 112); a feature of the urine in childhood, early adult life, and during convalescence from acute fevers. In non-albuminous and otherwise healthy urines cylindroids may be found, and are therefore not diagnostic of renal disease.

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* "Phila. Med. Jour.," Dec. 15, 1900.

† "Arch. f. Heilkunde," 1870, vol. xi, p. 130.

Floaters (*Clap Shreds*).—Congestion situated at any portion of the genito-urinary tract, preferably the urethra and adjacent structures, may cause the urine of such persons to contain small particles, or bodies, which are thrown down with the general sediment, or which, at other times, remain suspended in the liquid at different distances from its surface. The composition of floaters varies within wide limits. Guyon* distinguishes three varieties: (1) Those found at the top of the urine, composed of epithelium and mucus; (2) where a moderate amount of pus is added they sink some distance beneath the surface; and (3) where a preponderous amount of pus enters into their composition they sink to the bottom of the urine.

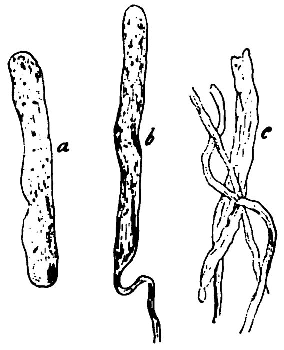

Fig. 120.—Cylindroids: *a*, *b*, Cast-like forms; *c*, filamentous forms (Ogden).

Spermatozoa.—These cells are to be found in the urine of men suffering from pathologic spermatorrhea, after intercourse, or emission the result of other excitants, and in the urine of women after coitus. When seen in the urine, they are found to retain their normal outline but are not motile, and in old urines the tails are detached from the heads. They stain readily with a solution of carbolfuchsin, and should be thus studied when question arises as to their general properties (see *Spermatozoa*, page 478).

Fragments of Tumors.—Small polypi from the bladder-wall are occasionally observed in the urine, while particles from sloughing surfaces, as of cancer, tuberculosis, or sarcoma of the bladder, furnish valuable aid to diagnosis. Malignancy of such structures as the vagina, rectum, and uterus may form fistulous communication with the urinary tract, and discharge their products with the urine. The diagnosis of renal tumor from the study of urine is difficult, and should not be made unless other conditions present warrant such conclusions by the surgeon. Cancer fragments are frequently derived from a leukorrheal discharge which has been added to the urine. Actinomycosis of the bladder is at times placarded by the appearance of the fungus in the urine (Fig. 121).

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* "N. Y. Med. Jour.," July 14, 1900.

Methods of Study.—Particles of tissue are best studied by teasing them with a fine needle, since they are usually too small for sectioning. In a case studied at the Philadelphia Hospital * the urine contained pieces of laminated membrane, scolices (heads of parasite), and hooklets—the products from a hydatid cyst (Figs. 122–125).

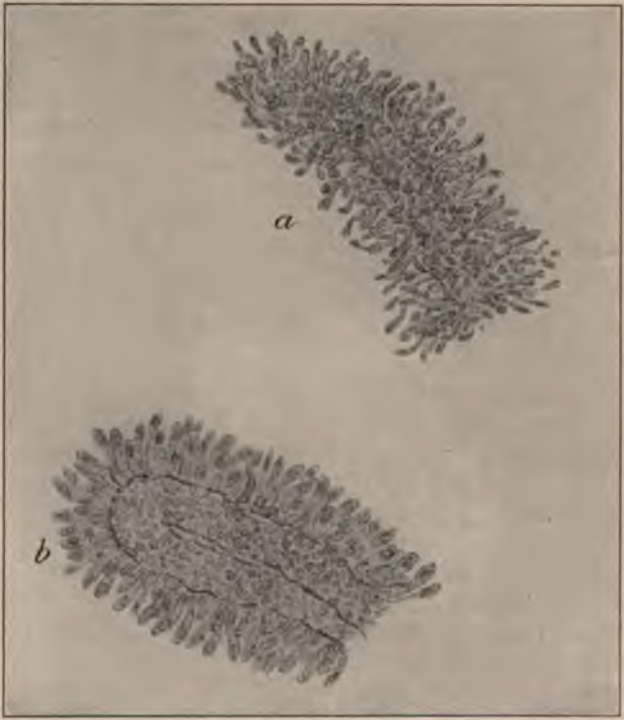

Fig. 121.—Portions of a villous growth of the bladder: *a*, Magnified 190 diameters; *b*, magnified 370 diameters (Ogden).

ANIMAL PARASITES.

Tænia Echinococcus.—This parasite rarely affects the urinary tract, yet its cysts may develop there and later discharge their contents with the urine. When the cyst is located in

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* "Amer. Med.," Jan., 1903, p. 20

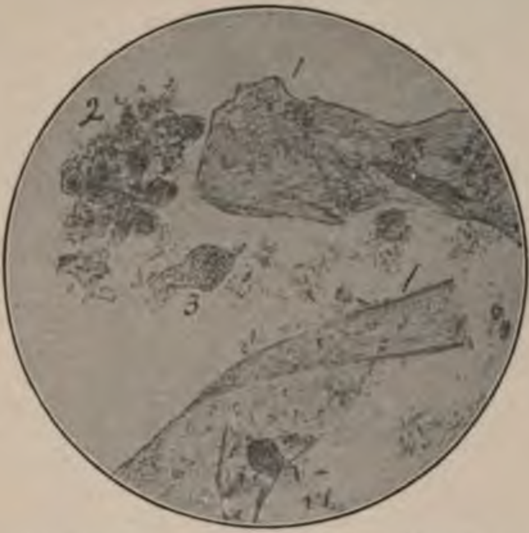

Fig. 122.—1, Shreds of membrane; 2, cluster of scolices; 3, scolex of Tænia echinococcus (obj. Queen two-thirds, eye-piece 4).

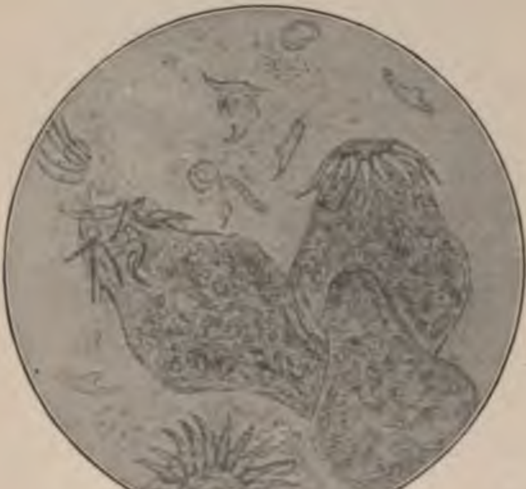

Fig. 123.—Scolices and hooklets, Tænia echinococcus, from urinary sediments (obj. Queen one-sixth, eye-piece 4).

the kidney's substance and communicates with the urinary tract, hooklets and scolices of the parasite in connection with shreds of the cyst membrane may be found in the urine, and in addition to these findings the urine commonly contains blood and casts, as was personally noted in a case studied in 1897 at the Philadelphia Hospital (Figs. 122–125). Cysts involving other viscera may form fistulous communications with the urinary tract, in which instance the usual products of the cyst appear in the urine.

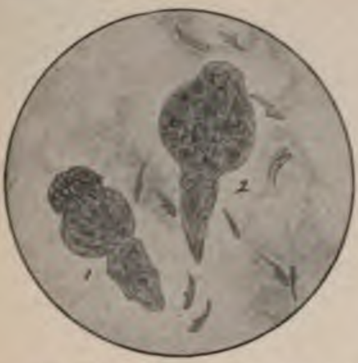

Fig. 124.—1, Scolex of Tænia echinococcus, showing crown of hooklets; 2, scolex and detached hooklets (obj. B. and L. one-sixth).

Filaria Sanguinis Hominis.—This parasite may appear in the urine of persons suffering from chyluria, or from hematuria of filarial origin. A similar case was studied by F. P. Henry. By way of repetition I studied the urine of an adult which showed hematoidin crystals and a parasite (Plate 17) of questionable classification. A surgeon removed one kidney from this

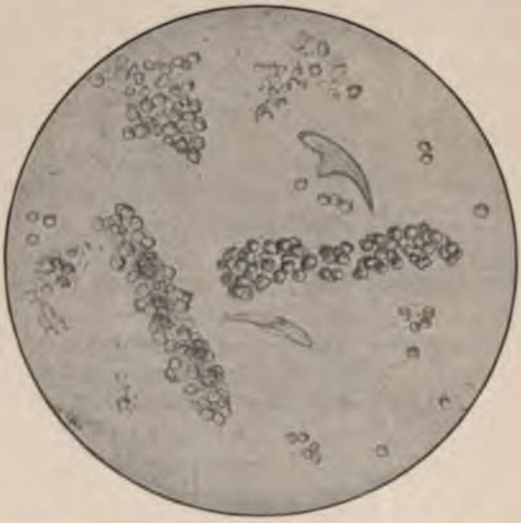

Fig. 125.—Urinary sediment showing blood-casts and hooklets of Tænia echinococcus (obj. Spencer one-sixth).

patient and reports that the pelvis and part of the kidney substance were occupied by a colossal abscess filled with creamy pus. The filaria is quite as easy of recognition when in the urine as when found in the blood, and presents all its characteristics, except that it is non-motile or its movements greatly lessened. Urine containing filaria may show blood, chyle, or pus, and for this reason it is often regarded as the etiologic factor in certain forms of tropic hematuria (Fig. 49). Blood falls to the bottom of the urine.

Schistosoma (Distoma) Hæmatobium.—The ova of this parasite are found in the urine of persons suffering from tropic hematuria, and were first described by Bilharz in 1851, who regarded them as an etiologic factor in the hematuria of Egypt. Harley, Brock, and others have studied this disease in the Cape of Good Hope and in the South African Islands, where epidemic hematuria is common.

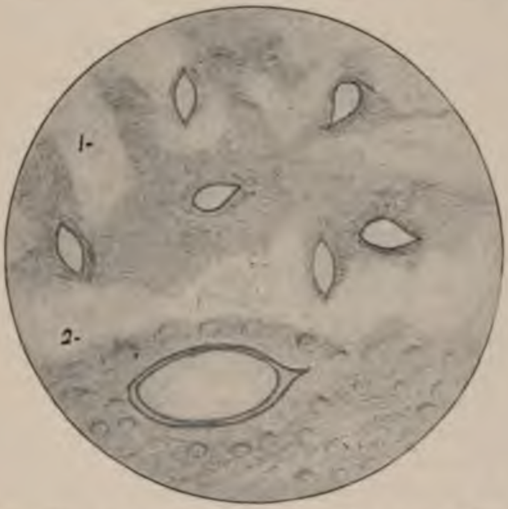

Fig. 126.—Schistosoma hæmatobium: 1, Ova in blood-clots from urine (obj. Spencer two-thirds, eyepiece 4). 2, Ovum shown under one-sixth objective.

Urine containing these ova is apt to display a heavy sediment, which when studied under a low-power (two-thirds) objective is found to be composed largely of blood-clots, and it is in these clots that the eggs are found most abundant (Fig. 126). At times it is necessary to tease the clot in order to liberate the ova from the mass; and after such treatment one often finds ova floating in the urine. To preserve these ova as permanent specimens the usual method employed for urinary sediments will be found of value (see *Mounting of Casts*, page 259).

The fully matured ovum is the shape of a hen's egg, $\frac{1}{175}$ to $\frac{1}{150}$ inch in length, and about $\frac{1}{325}$ to $\frac{1}{300}$ of an inch in breadth. An irregularity in size obtains even in mature specimens; some eggs being found narrow and long, while others are distinctly oval. The most immature ovum presents a hyaline envelop with a short spine (Plate 19A), and by careful focus is seen to contain granules of various sizes. Older specimens show segmentation in different stages, and by close study of such ova one may trace the various developmental stages, step by step, from a mere granular mass until the embryo attains a definite structure (Plate 19A). The capsule is sufficiently transparent to permit the study of its contents. At the narrow end it is continued as a short, sharp spine which has an average length of $\frac{1}{2000}$ inch.

"The embryo, until the time draws near for its obtaining liberty, lies quiescent in the shell, only now and then moving its head from side to side, or occasionally drawing its whole body upward with a jerking movement" (Brock).* The cephalic and caudal extremities of the embryo are readily distinguished when under a one-sixth objective, the former being commonly directed toward the spine. Later the body of the embryo appears to be surrounded by cilia. The time required for the hatching of these embryos after they are voided with the urine varies from a few hours to several days. The capsule becomes thin and develops a longitudinal slit through which the embryo escapes (Plate 19A). When the urine above the sediment has been decanted and replaced by water, the embryos are to be seen swimming actively in this liquid (Brock). Through the courtesy of Dr. Claude A. Smith, of Atlanta, I was provided with urine containing many of these ova and was able to confirm the observations of Brock, as shown by the accompanying illustrations. The empty capsule is to be found floating in the liquid after the escape of its embryo.

The further development of the embryo outside of the body and its mode of re-entering the system are not fully determined. The fact that bilharzia affects mostly males before the age of eighteen, a time when they bathe frequently in the streams of infected districts, as well as the additional evidence that women and girls of these districts are rarely known to bathe in the streams and are practically free from Bilharz disease, offers evidence favoring the parasites gaining access to the body during the act of bathing. It is possible that they may ascend the urethra, for it is to be remembered that the mature worm finds a resting-place in the

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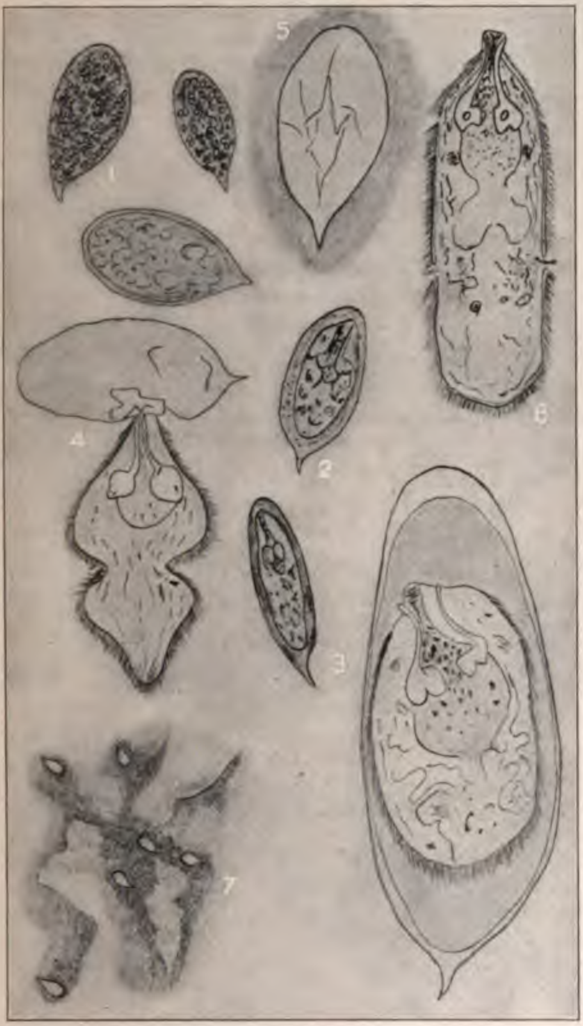
* "Jour. of Path. and Bact.," Edinburgh and London, Oct., 1893.

PLATE 19 A.



SCHISTOSOMA HÆMATOBIUM.

1–4, Various stages in development of embryo; 5, empty shell; 6, surviving embryo (after Brock); 7, ova in urinary sediment (personal observation).

venous systems of the bladder or abdominal viscera, and her ova may escape with the urine, the feces, or the sputum.

Oxyuris Vermicularis.—This parasite may in rare instances appear in the urine, either as a result of abnormal communication between the urinary tract and the rectum, or in the case of children, where the parasite enters the vagina or the urethra, in which instance the symptoms of acute inflammation of these parts are noted (see *Vaginitis*, page 484). In one case of vaginitis I found the urine to contain many ova and two specimens (females) of the oxyuris.

Ascaris Lumbricoides.—The ascarides or their ova are seldom found in the urine, and occur only as the result of fistulous communication between the rectum and the urinary tract.

Rhabditis Genitalis.—The name given by Scheiber to a variety of worms which he found in the urine, believing the affection to originate from the genito-urinary tract. I have observed the following described parasite in two cases of hematuria * seen at the Pennsylvania and Philadelphia Hospitals respectively. Dr. Ch. Wardell Stiles, of the Bureau of Animal Industry, at Washington, declined to make a specific determination of this parasite. The patient, a male of sixty-one years, presented nothing abnormal for one of his age, and gave no history of having ever before voided bloody urine. On rising in the morning he noticed that his urine resembled blood, and it remained unchanged during the day. He suffered no discomfort, and during the day did not void urine oftener than usual. A specimen taken from that passed on retiring and during the night following was also bloody and contained a few parasites. In the morning the urine was normal in appearance, did not contain parasites, and the centrifugated sediment showed but few blood discs. The urine, on standing, displayed a heavy sediment, a microscopic study of which showed it to be composed, for the most part, of blood-cells, clots, few epithelial cells, and many blood-casts. Everywhere the sediment was found to contain many small worms, which are best described by the accompanying illustration (Fig. 127). A drop of this sediment, when studied under a two-thirds lens, often presented from two to six of these parasites in a single field of the microscope. Their movements consisted in coiling and uncoiling, apparently reaching forward with their tapering extremity—these movements being repeated in such rapid succession that they were enabled to travel across the field of the microscope in a few seconds. The serpentine movement, as shown by the embryos of the filaria, was not observed. They remained alive for twenty-four hours in a specimen of urine that had been placed in an incubator at a temperature of 37° C.; but when kept at room temperature they were found motionless after a few hours. In the urine there appeared to be great variation in the size of these worms, as is shown by the following measurements: Total length, 0.036 to 0.053 inch; diameter at greatest part, 0.003 to 0.0035 inch; diameter at the junction of the greater one-fourth with the remaining three-fourths of the body, 0.002 to 0.0025 inch.

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* "Amer. Med.," Jan. 3, 1903, p. 20.

During my term of service at the Pennsylvania Hospital (1901) I studied a specimen of bloody urine, voided by a male patient, in which many parasites apparently identical with those observed in connection with the previous case were found.

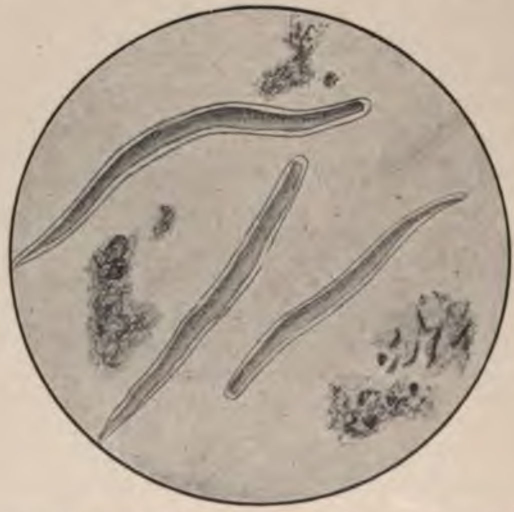
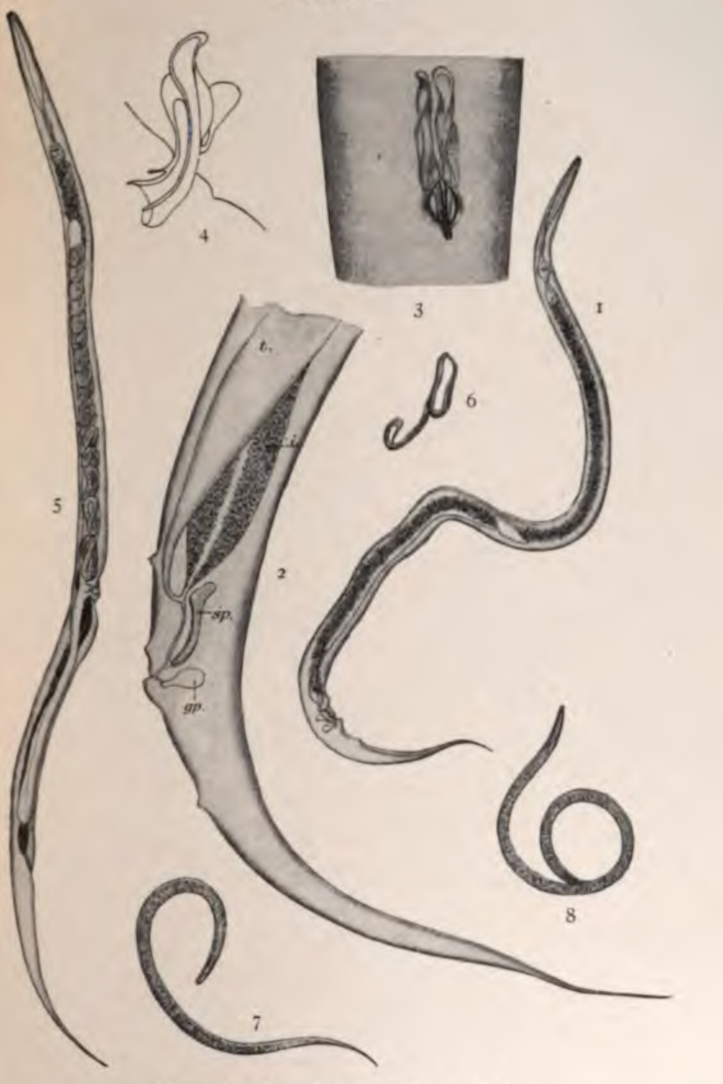


Fig. 127.—Parasites found in bloody urine (obj. B. and L. one-sixth).

Trichocephalus Dispar.—Ova of the Trichocephalus dispar were present in the urine of both patients (Fig. 171).

Anguillula Aceti.—Few instances are recorded where the urine has contained this parasite, and while its detection is most unusual, it is necessary that every microscopist be ever alert concerning the A. aceti or "vinegar eel." The A. aceti (Plate 20) thrives in both alkaline and acid urines. The average size of the parasite is 1.2 mm. by 0.033 mm.; while that of the female is about 1.9 mm. by 0.06 mm., and few specimens are seen to reach 2.25 mm. in length. The uterus of the mature female is distinct and contains two or more ova and less often embryos. The young

PLATE 20.



Vinegar Eel (Anguillula Aceti).

1, Lateral view of male specimen of the vinegar eel (*Anguillula aceti*) from the man bladder (greatly enlarged).

2. Caudal portion of same species: *i*, Intestine; *t*, testicle; *sp*, spicule; *ap*, cessory piece (greatly enlarged).

3. Ventral view of cloacal opening of same, showing partially extruded spicules reatly enlarged).

4. Outline lateral view of partially extruded spicules (greatly enlarged).

5. Gravid female vinegar eel from the human bladder. Embryos and eggs e present only in the anterior horn of the uterus (greatly enlarged).

6. Young embryos of same (greatly enlarged).

7, 8. Somewhat older embryos (greatly enlarged). The esophagus is evident both specimens (Stiles).

forms may vary in length from 0.25 to 0.7 mm. The male worm is provided with a pair of thick, double, curved spicules on the ventral surface (Plate 20, Fig. 3), and projecting from each is a fan-shaped piece of fin. I have studied the urine from two patients, one a male, in which the vinegar eel was present. The woman had been taking vaginal douches containing vinegar.

Detection.—It is easy to obtain this parasite from table vinegar and thus conduct comparative studies. It resembles closely the Strongyloides intestinalis (see page 419), which is found in the feces, Thayer* having reported two American cases. Stiles has offered the following—

KEY TO CLINICAL DIAGNOSIS OF WORMS IN THE URINE AND IN THE VAGINA.

EGGS.

1. Eggshell thick, ellipsoid, 64 to 68 μ by 40 to 49 μ, with mosaic structure; embryo not developed; indicates infection of kidney...*Dioctophyme renale.*
Eggshell ovoid, 135 to 160 μ by 55 to 66 μ, without mosaic structure, usually with sharp spine; contains ciliated embryo; indicates infection of blood-vessels with trematodes (Egyptian hematuria, bilharziosis) *Schistosoma hæmatobium*, page 295.
Eggshell thin, oblong, 50 to 54 μ by 20 to 27 μ, contains elongated worm; indicates infection of rectum with pinworms not found in the urine of male patients ....*Oxyuris vermicularis*, page 414.

EMBRYOS.

2. Embryo ciliated (bilharziosis)........*Schistosoma hæmatobium*, page 295.
Embryo not ciliated elongate..3
3. Esophagus distinct, with posterior bulb armed with chitinous teeth (rhabditiform embryos) 4
Esophagus not very distinct, no posterior bulb; same embryos also found in the blood, 270 to 300 μ long........*Filaria sanguinis hominis*, page 147.
4. Embryo 140 μ long; adults 3 to 12 mm. long in rectum *Oxyuris vermicularis.*
Adults in urine, but not in rectum......................................8

LARVÆ AND ADULTS.

5. Elongated and flat; tape-worm larvæ..........*Sparganun Mansoni.*
Body round..6
6. Body large, may attain 40 to 100 cm. in length; usually red in color *Dioctophyme renale.*
Body less than 12 mm. long, whitish....................................7
7. Body 3 to 12 mm. long; male with single spicule; same parasite found in rectum....................................*Oxyuris vermicularis.*
Body less than 3 mm. long; male with two spicules and accessory piece......9
8. Male without caudal bursa..........................*Anguillula aceti.*
Male with caudal bursa..............................*Rhabditis pellio.*

Eustrongylus Gigas.—Evidence of this parasite is at times manifest in the urine and may be accompanied by chyluria or hematuria, Moscato recording a case of the former, and Clark one of the latter. Dr. John A. McKenna, of Philadelphia, has detected the ova of this parasite in the bloody urines of two children. Portions of the adult worm were obtained from one case in urine drawn by catheter.

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* "Jour. of Exp. Med.," Nov. 29, 1901.

Bothriocephalus Liguloides.—Leuckart has recorded an instance occurring in eastern Asia where this parasite was found in the urine of man.

Amœba Urogenitalis.—Baelz[*] described under this caption large, actively motile amebæ found in the urine and vaginal secretion of a female aged twenty-three years, who was at that time suffering from pulmonary tuberculosis.

Infusoria.—Urine may be found to contain these bodies when it has been allowed to stand for a time and is faintly alkaline in reaction. They have no known pathologic significance. The Trichomonas vaginalis (Fig. 146) has been detected in the urine by Marchand, Muira, and Dock.[†] The author has studied bodies in the urine closely resembling the cercomonads found in the feces.

VEGETABLE PARASITES.

Fungi.—Normal urine when voided contains neither fungi nor bacteria in appreciable numbers, but when allowed to stand in a warm place these organisms soon develop (ten to twenty-four hours). They may be divided into molds, yeasts, fission-fungi, and bacteria, of which we find for the most part non-pathogenic forms. Urine undergoing ammoniacal change presents fission-fungi in great numbers and occasionally yeasts. Molds are not unusual when the urine contains sugar; but where alcoholic fermentation has ceased this fungus develops with rapidity. Molds may form a thick surface growth which at first floats, but later may sink to the bottom of the liquid. Such urine is commonly turbid and in addition contains yeasts and numerous bacteria.

Clinical Significance.—A copious growth of the yeast fungus in the urine is highly suggestive of glycosuria.

Microscopically, decomposing healthy urine presents a variety of pictures; and these changes are doubtless produced by a number of organisms, among which micrococci are given a prominent place, appearing on the surface in pure cultures (Micrococcus urea). This coccus is of large size and often forms in short chains. Bacilli of variable size, some containing spores, others in clusters and chains, of both motile and non-motile varieties, are also present in such sediments, together with cocci of various sizes, arranged singly, in pairs, and in clusters. Sarcinæ of small size are occasionally met with in such urines (Fig. 138), and I have found

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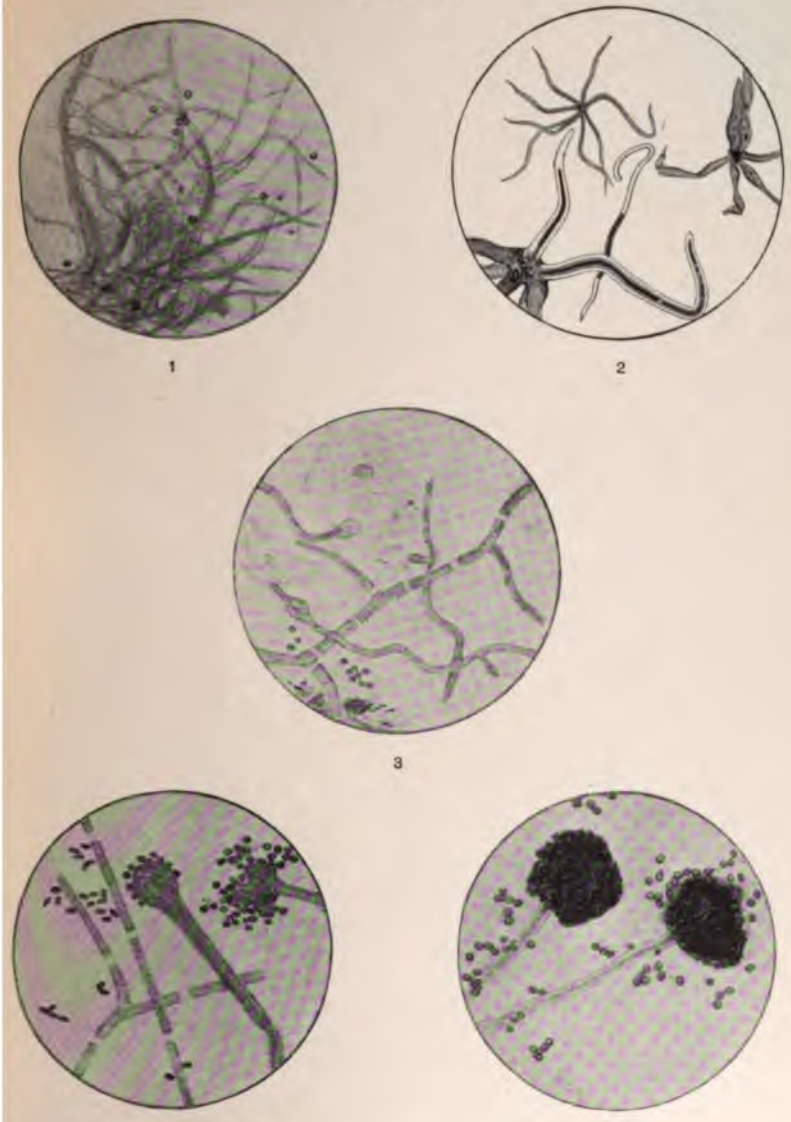
[*] "Berl. klin. Woch.," 1883, vol. xxiii.

[†] "Amer. Jour. Med. Sci.," Jan., 1896.

PLATE 21.



ASPERGILLUS.

1. From cultures of aspergillus fumigatus on acid urine (obj. Queen one-sixth; eye-piece 2).

2. Peculiar arrangement (octopus formation) of fungous growth. At lower left quadrant is a portion of the fungus (highly magnified).

3. From urine during an attack of cystitis (obj. Queen one-sixth; eye-piece 2).

4. From urine (diabetic) during an attack of cystitis (obj. Queen one-sixth; eye-piece 2).

5. Aspergillus niger from culture on acid (diabetic) urine (obj. Queen one-sixth; eye-piece 2).

them in the sediment of fresh urines prior to the development of fermentation.

Actinomyces.—Actinomycosis is a rare disease of the genito-urinary tract, but where involvement of these organs exists, it is likely to be placarded by the appearance of the ray fungus or its products (mycelia) in the urine (Fig. 183).

Aspergillus.—Renon observed spores of the Aspergillus fumigatus in the urine of animals suffering from experimental aspergillosis.* The author has found both spores and mycelia of the Aspergillus fumigatus present in the urine of acute cystitis, and in cystitis occurring in a diabetic (Plate 21); and has conducted a series of cultures studying the effect of both the A. niger and A. fumigatus on urines.†

Bacteriuria.—A urine containing many bacteria when voided is to be regarded as a serious symptom even though the individual organisms present are not classed as pathogenic in nature; yet such a condition of the urine may persist over a long period without other appreciable evidence of disease (idiopathic bacteriuria). Theoretically, bacteriuria should always ensue as a result of bacteremia. The urine contains specific bacteria during the course of certain infectious maladies.

Method of Obtaining Urine.—Urine for bacteriologic study must be collected under aseptic precautions lest the result of our study be absolutely nil. To obtain urine from the female bladder, cleanse the vestibule of the vagina and mouth of the urethra by using first a solution of mercuric bichlorid (1 : 1000) followed by a solution of boric acid and later by sterile water. Second, a glass catheter that has been previously sterilized by boiling, and placed in sterile water, is now lubricated with sterile olive oil or glycerin and introduced through the urethra into the bladder. Third, allow the urine to flow off through the catheter for a few seconds. Then permit it to flow into sterilized test-tubes or into a sterile bottle, and cork with sterilized cotton.

Caution.—Incline the open tube while the urine is entering it to prevent air-borne bacteria from falling into the tube. Cork the tube or the bottle with the cotton stopper before placing it in the vertical position. Cultures may be made from urines thus obtained, and in my experience it has been rare to find such specimens contaminated by extraneous organisms.

The same necessary precaution should be observed in obtaining urine from the male bladder, using a metal or rubber catheter.

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* "Comptes Rendus des Seances et Memoiries de la Societe de Biologie," Feb. 9, 1895.

† "Phila. Hosp. Rep.," 1900.

It is my practice to force bichlorid-of-mercury solution (1 : 1000) through the rubber catheter and then to immerse it in this solution of bichlorid for a short time, which renders the chances of contamination by the catheter infinitesimal.

The collection of urine from the kidneys by catheterization of the ureters requires, in addition, the assistance of the surgeon, as do also methods of segregation.

Making Cultures.—The making of cultures and identifying micro-organisms are those commonly in vogue, and for the latter of these the reader is referred to special works upon bacteriology.

1. Take three culture tubes containing about 20 c.c. each of agar-agar and heat them (by standing in warm water) sufficiently to liquefy the medium.

2. Label these tubes (*a*), (*b*), (*c*).

3. Shake the urine well and add to tube (*a*) one loop (one drop) of urine, stirring with the platinum needle (Fig. 197) to mix it well through the medium and pour into a Petri dish. To tubes (*b*) and (*c*) add two and four drops respectively, and proceed as in the case of (*a*).

4. Place plates in a cool place until the medium has solidified; then place in an incubator (temperature, 37.5° C.) for from twenty-four to forty-eight hours. Cultures and smears should be made from such colonies of growth as may develop, transferring the cultures to several forms of medium.

5. In case a bacteriologic study is made at stated intervals the number of colonies developing from tubes (*a*), (*b*), and (*c*) respectively should be counted, and in this way the degree of bacteriuria estimated.

It is also well to make several slant inoculations on agar-agar, Loeffler's blood-serum, and on bouillon-peptone solution, special media being necessary for the gonococcus, tubercle bacillus, and bacillus of influenza.

Staining of Bacteria.—Fill two or more of the special tubes of the centrifuge with the urine and centrifugate for five minutes; then remove the tubes and lift the sediment thus collected into a pipet and transfer a small drop of it to the center of a clean slide which has been arranged in the slide forceps (Fig. 186); spread to form a thin film, dry by gentle heat, and stain for two minutes with carbolfuchsin or 5 per cent. aqueous solution of methylene-blue.

Caution.—Remove the stain by allowing a feeble current of water to fall upon one end of the slide and to flood gently over the specimen.

The specimen may be dried over a low flame or in the air and mounted in Canada balsam. Study by the direct method consists in adding a cover-glass to the stained sediment before drying. Much time and labor may be saved by adopting this latter method, and by it specimens may be satisfactorily viewed under a one-twelfth oil-immersion objective. The staining of the tubercle bacillus and of the gonococcus will be given in detail in conjunction with the study of sputum and urethral pus respectively, and allusion will be made to some of the bacteria to be encountered in the urine.

Tubercle Bacillus.—This organism is found in the urine when ulcerative or suppurative tuberculous lesions involve or communicate with the urinary channel. In this situation the bacilli are commonly found in clusters and often intimately associated with shreds of sloughed tissue (Plate 30). Isolated bacilli are not common, yet such findings may occur in connection with miliary tuberculosis, with or without ulcerative lesions of the genito-urinary tract; and according to Frick and Walsh, tubercle bacilli are to be found in the urine of a large percentage of cases of pulmonary tuberculosis (see *Staining of Urinary Sediment*, page 258). Personally, I have studied over 1000 specimens of urine for tubercle bacilli and have found this organism but three times, though other acid-fast bacilli have been common findings. It is necessary to differentiate between the tubercle bacillus and the smegma bacillus (see page 441). Detection of the tubercle bacillus in the urine by means of cultures may at times be necessary, but is scarcely practicable; a more practical method of diagnosis where the bacilli are not found and the clinical features suggest strongly their presence, is to inoculate a guinea-pig or a rabbit with a few drops of the suspected urine into the chamber of the eye, into the peritoneal or the pleural sacs, or subcutaneously, in which case the animal will develop tubercular retinitis, peritonitis, or pleuritis respectively. There is no form of inoculation with the tubercle bacillus more virulent to guinea-pigs than when the bacilli are contained in the urine.

Gonococcus.—Gonococci may be present in the urine during the course of gonorrheal urethritis, at which time the organism stains with the usual anilin dyes (see page 495). I have found it difficult to detect beyond question of doubt the gonococcus in urinary sediments; and the differential staining by means of Gram's solution has been unsatisfactory, since I have not been able to make a positive diagnosis of gonorrhea through a microscopic study of the urine alone.

Caution.—The urine of women not infrequently contains a diplococcus which, from its staining properties alone, is indistinguishable from the gonococcus, and the identity of this organism is impossible except where cultural studies are conducted. The urine may under certain conditions modify the growth of the bacteria, and experience has forced me to regard bacteriologic study of the urine as of limited value when in reference to the gonococcus.

Ulcerative Endocarditis.—Here, too, the urine at time contains the specific bacterium acting as a causal factor in the production of the endocardial lesion. Such study is impracticable since a study of the blood furnishes more conclusive evidence.

Septicemia and Pyemia.—The urine in these conditions may rarely contain clumps of pathogenic cocci, and seldom these bodies are arranged in the form of casts which appear to be formed of bacteria. It is to be borne in mind that at such times all vital structures are much reduced, thus forming a suitable soil for bacterial developments along the urinary tract, and that these bacteria, in part at least, may not enter the urine through the kidneys.

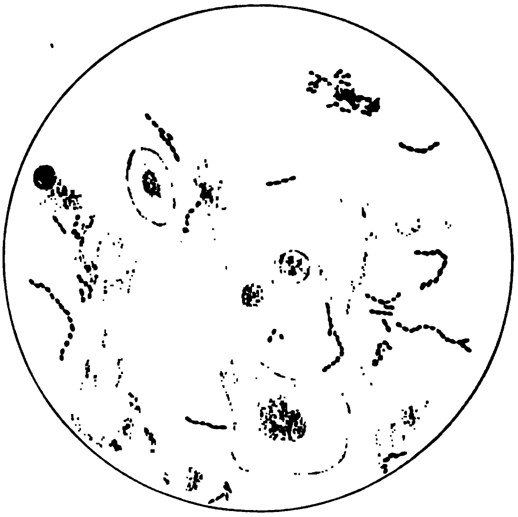

Fig. 128.—Streptococcuria; case of a male of eighteen years; history of albuminuria for six months. Urine also contained few cylindroids and hyaline casts (obj. B. and L. one-twelfth oil-immersion).

Acute Nephritis.—Many writers have found cocci in the urine of this affection, and bacilli are reported as occurring in the nephritis of children; but as yet their diagnostic value is restricted. In the acute nephritis complicating pneumonia, diplococci have been found. The theory of bacteriuria in the nephritis of acute infectious fevers appears plausible and has been ably exemplified in a number of instances; *e. g.*, typhoid fever. The accompanying illustration (Fig. 128) was made from the urine of a youth who was suffering from subacute nephritis.

Typhoid Fever.—Typhoid bacilli are described as occurring in the urine of persons suffering from this disease by Newmann,* who found the organism present in six of twenty-three cases studied. Wright and Semple found typhoid bacilli in six of seven cases studied.† Gwynn has made an exhaustive study of the subject,‡ from which he concludes that typhoid bacilli appear in the urine of typhoid patients in 30 per cent. of the cases; and since this report there have followed a number of reports, most of which have been confirmatory of Gwynn's observations, few finding these bacilli present in a higher percentage of cases.

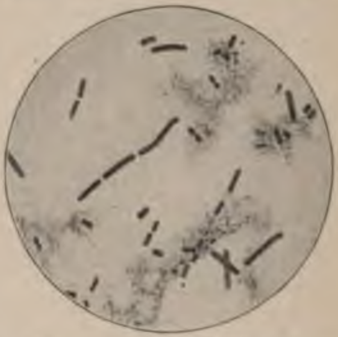

Fig. 129.—Typhoid bacilli in urine. Third week of disease. Stained with carbolfuchsin (obj. B. and L. one-twelfth oil immersion).

Clinical Significance.—Typhoid bacilli may occur early in the course of the disease, but are more commonly present by the third week; they persist during convalescence and often for a long time thereafter, and are present in the urine of both severe and mild cases of enteric fever. Urine containing typhoid bacilli displays a fine clouding which lends a light and finely flocculent appearance to the fresh specimen. This, however, is not constant, and the urine may present the usual febrile characteristics where many bacilli are present (cloudy, dark color, and a heavy sediment). Cultures are often pure, and smears made from the urine's sediment show many bacilli (Fig. 129). Richardson concludes that the percentage of cases in which typhoid bacilli appear in the urine bears a somewhat similar relation to the percentage of cases where the bacilli are present in the blood. The presence of typhoid bacilli in the urine may be influenced greatly by the administration of certain drugs, as urotropin, and by local treatment directed to the bladder. Their presence bears no relation to the severity of the disease, and is not influenced by the presence of complications or sequelæ. The presence of large numbers of these bacilli is suggestive of the disease in the absence of other valuable symptoms and signs.

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* "Berl. klin. Woch.," 1888, vol. xxv, Nos. 7 and 9.

† "Lancet," July 27, 1895.

‡ "Johns Hopkins Hosp. Bul.," June 18, 1899; Phila. Co. Med. Soc., Oct. 24, 1900.

Identity.—Where these bacilli are found to decolorize by Gram's method of staining, and to present the morphologic characteristics, as well as the peculiar agglutination reaction with the patient's serum (Widal reaction), it will be safe to determine the presence of typhoid bacilli in the urine in typical cases.

Reaction.—Such urine commonly gives an acid reaction when voided, and may even remain acid after standing for a long time. The number of bacilli present in the urine varies greatly.

Erysipelas.—In this disease, where complicated by acute nephritis, the urine has been found to contain organisms indistinguishable in form from the Streptococcus pyogenes erysipelatis of Fehleisen. In the study of a large series of cases at the Philadelphia Hospital (54 in all) I was unable to recover such streptococci from the urine, though a few of these urines contained a trace of albumin. Non-pathogenic streptococci were present in some of the specimens.

Glanders.—In 1885 Philipowicx* detected the bacillus of glanders in the urine of a man suffering from this disease.

Relapsing Fever.—The spirillum found in the blood during the paroxysms of this disease appears in the urine when hematuria obtains, and during the febrile exacerbations the urine has been found to contain many bacteria.

Colon Bacillus.—This organism (bacilli members of the colon group) is normally present in the urethra, and becomes numerous in the urine in cases of urethral and of vesical congestion, urethral stricture, prostatitis, and during constipation (see *Acute and Chronic Cystitis* and *Pyelitis*). The bacillus of Sternberg and other members of this group have been recovered from the urine of yellow fever, but their presence has not been shown to be of clinical significance.

Diphtheria.—The Klebs-Loeffler bacillus occurs in the urine when the diphtheric lesion is situated so as to communicate with some portion of the genito-urinary tract; and since this bacillus enters the circulation in malignant types of infection (see page 450) its recovery from the urine in such instances is to be anticipated.

Scarlet Fever.—Here, also, the urine may contain many microörganisms; as streptococci, bacilli, diplococci, and micrococci. I have found it difficult to show any relation existing between such organisms and the disease in question. Class has found the "Diplococcus scarlatinæ" in pure cultures in the kidney's substance of animals inoculated with this organism,* and has also cultivated this diplococcus from the urine of persons suffering from scarlet fever who presented scarlatinal nephritis.

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* "Wien. med. Blat.," vol. xxxiv, pp. 673 and 710.

Acute Cystitis.—The reaction of the urine will be found to vary according to the bacterium present. Probably 50 per cent. of cystitic urines will show infection with the Bacillus coli communis (commonly in pure cultures) and the degree of acidity high; while in a staphylococcous infection the acidity is not decided. The Staphylococcus pyogenes albus occurs in from 15 to 25 per cent. of cases, and far less often the Staphylococcus pyogenes aureus is found. The Bacillus pyocyaneus when present is placarded by the greenish tinge it lends to the urine, which color (green) increases if the urine is kept at incubating temperature. T. R. Brown † reports a case of infection with this organism, and I have found it in the urine of acute cystitis twice; once in pure cultures.

The Bacillus proteus vulgaris has been found by Brown in alkaline (ammoniacal) urines containing both blood and pus.

Chronic Cystitis.—In the study of 24 cases Brown ‡ recovered from the urine Bacillus coli communis eleven times, and once the Bacillus tuberculosis; Staphylococcus pyogenes aureus three times; Staphylococcus pyogenes albus twice; and in two cases pyuria existed with sterile urine. In seven cases of cystitis with pyelitis the Bacillus coli communis was recovered four times (urine acid); Bacillus proteus vulgaris once, and Staphylococcus albus twice (alkaline or acid alternating).

Acute and Chronic Pyelitis.—In nearly 50 per cent. of cases in which urine is obtained by catheterization of the ureters the colon bacillus will be found, in which instances the urine is acid. The Bacillus proteus vulgaris figures in from 20 to 30 per cent. of cases (alkaline urine). Where the process is tuberculous, the Bacillus tuberculosis is to be found by staining and by inoculation of animals with the suspected urine. Cultivation of this organism from the urine is impracticable.

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* "Proc. Path. Soc. Chi.," May, 1899.

† "Johns Hopkins Hosp. Rep.," vol. x, Nos. 1, 2.

‡ *Ibid.*, vol. x, Nos. 1, 2.

CHAPTER III.

GASTRIC CONTENTS.

THE contents of the stomach is composed of the fluids secreted by the pyloric and by the cardiac glands of the stomach, which provide its active digestive ingredients: it also contains a portion of the buccal secretion which has gained access to the stomach through swallowing, but at the time when we recover the stomach-contents it is much altered, due to the digestive action of the fluids secreted by the stomach-glands.

METHOD OF COLLECTION.

The Stomach-tube.—The stomach-contents is best collected by the use of the stomach-tube, which is but a modification of the tube first devised and employed by the late Dr. Philip S. Physick, then Professor of Surgery in the University of Pennsylvania. Physick published a description of his original device in October, 1812, in the "Electrical Repertory," vol. iii, p. 111; but this instrument had been used by Dr. Physick since its first employment in 1800, when, so far as the records show, he first emptied the stomach of its contents by this method, and introduced into the stomach stimulants to patients from whom he had removed poisonous drugs. In 1803 Dr. Physick had made, in Paris, a special tube for the evacuation of the stomach. Julius Friedenwald* has published an interesting historic note on this instrument.

This tube should be at least 75 inches in length, and provided at one extremity with three fenestra: one at the end of the tube, the other two at its side, about one and two inches respectively from the tip. The other extremity of the tube is expanded into a funnel, the base of which is from three to four inches in diameter, and the portion of the tube between the funnel and the fenestra is of a constant caliber of about one-half inch in diameter. Many operators prefer a tube in sections anchoring the one extremity to a glass funnel. The accompanying cut (Fig. 130) will illustrate the tube with which I have had the most satisfactory results.

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* "Johns Hopkins Hosp. Bull." Sept., 1903.

Boas has devised a special bulb, which is usually located midway between the portion of the tube showing the black circle and the funnel; but where the sole object is merely to evacuate the stomach, I have found this bulb of no service. In fact, a tube with an expanded portion between the funnel and the fenestra is often most annoying, and I have never found this expansion of any practical value. The foregoing statements are not to be confused with the employment of the stomach-tube for the ascertainment of the capacity of the stomach or for the purpose of distending this viscus and thus outlining its position; in the latter of which instances air must be forced into the stomach, but the technic for such procedure is not within the scope of this volume.

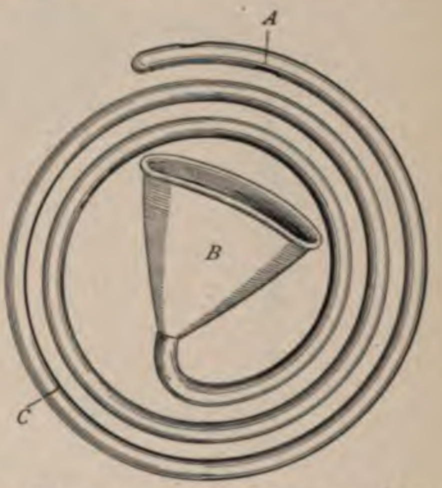


Fig. 130.—Stomach-tube: *A*, Fenestra near tube's end; *B*, funnel-like expansion; *C*, mark showing distance to be introduced.

Counterindications.—Among the counterindications to the introduction of a tube into the stomach are: Valvular heart disease where compensation has been lost; thoracic aneurysm; angina pectoris; pulmonary or gastric hemorrhage; severe bronchitis; arteriosclerosis of high grade; and the various febrile diseases, including pulmonary tuberculosis. The introduction of a tube into the stomach in this latter condition is attended with a certain amount of risk, due to the fact that tuberculous ulceration of the stomach, while rare, may exist and the organ be perforated. Perforation results from extreme retching, rather than from the instrument itself. In one such case I have seen rupture of the stomach occur, and I can recall two instances where the stomach-tube was passed directly into an aneurysm.

Introduction.—1. Place the tube in a bowl containing warm water; elevate its funnel end at a distance of about two feet above the bowl, and pour through the tube at least one pint of warm water; then allow the entire tube to remain coiled in the bowl of warm water.

2. In case the patient is not confined to bed, direct that all clothing around the chest and abdomen be loosened so that the

epigastrium is covered by a single layer of clothing composed of a thin garment. The patient is now directed to sit comfortably in a chair or to be pillowed up in bed; where there is some objection to the patient sitting, direction should then be given to have him lie on his left side. Place a heavy towel over the patient's chest, and pin it at the back of the neck in order to prevent the saliva, which always flows freely from the mouth while the tube is in position, from soiling the clothing or causing the patient discomfort.

3. Most important in the introduction of the stomach-tube is it to obtain the patient's confidence and to assure him, without hesitancy, that the instrument will not harm him, and that the slight smothering sensation and nauseating effect which it always excites are increased by his own effort; and that if he will try to control the spasm of his throat, you will be able to introduce the tube more easily, and that the choking sensation will be materially lessened. This step performed well, all difficulties are ended and the stomach-tube is introduced with comparative ease in the most hysteric persons; neglected, it is always introduced with difficulty.

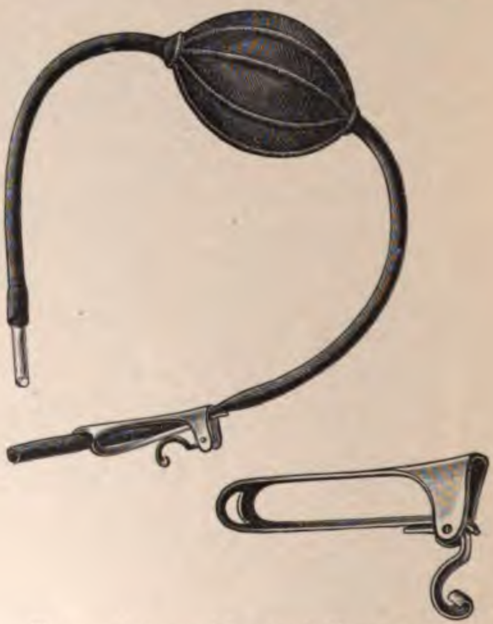


Fig. 131.—Aspirator of Boas (one-half natural size).

4. Remove the tube from the water, and without drying grasp it between the thumb, index-, and second fingers (Fig. 132). Direction should be given to the patient to open the mouth and to lower the chin slightly, when introduce about six inches of the tube, passing it directly over the tongue to the back of the throat. The patient is now directed to swallow, and the tip of the tube will immediately enter the esophagus. Pass the tube gradually by the thumb and finger, taking care to exert force only at the time when the patient swallows—and he should be directed to swallow as often as possible. Continue this introduction until the circle-mark on the tube (Fig. 130) reaches the patient's lips; then hold the tube in position by the left hand and drop the funnel into the receptacle for the fluid. At this point in the operation direct the patient to bend forward and to endeavor to contract and relax his abdominal muscles; or to cough slightly three or four times in succession. Should these

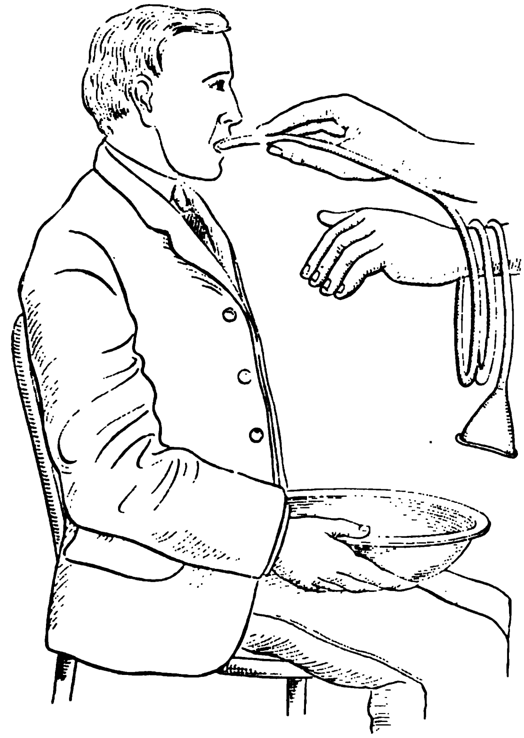


Fig. 132.—Position of the patient for introduction of stomach-tube; also method of passing tube into the mouth.

efforts fail to expel the stomach-contents, the operator should place his left hand over the epigastrium and exert gentle pressure while the patient repeats the expulsive efforts previously described.

In rare instances it is impossible to recover any fluid in this manner, when it is necessary to have a glass containing just sufficient water to fill the tube from its funnel to its tip. Elevate the funnel and pour this quantity of water in it, and at the moment all the water enters the tube, the funnel is dropped into a clean bowl and the expulsive efforts repeated both by the patient and by the operator (Fig. 133) The water that was introduced into the funnel will be expelled, and is likely to be followed by a free flow of the gastric contents. In order to prevent the gastric fluid from being diluted with the water the funnel is placed in a second receptacle

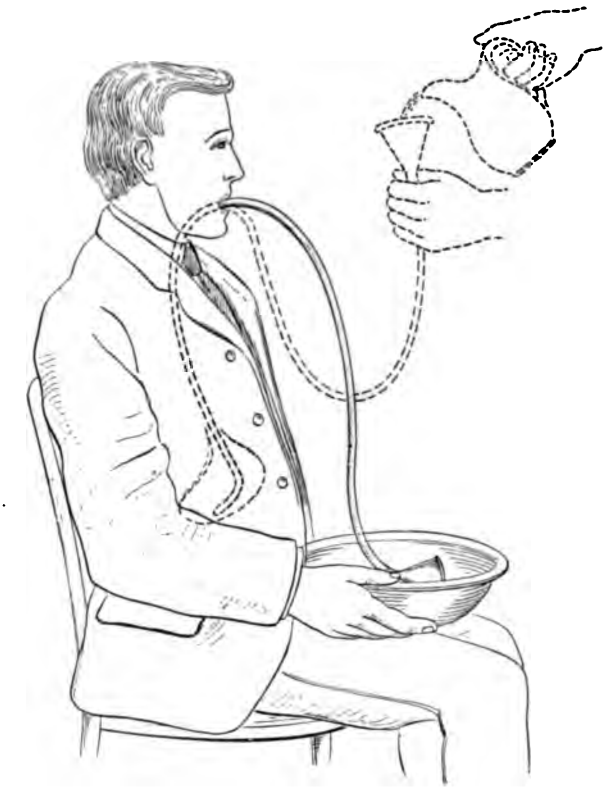


Fig. 133.—Method of inducing expulsion of gastric contents by siphonage, as directed in the text.

as soon as the water has escaped; thus the gastric juice will be a correct representative of the stomach's secretion. After the gastric fluid has started to flow, it is not likely to be checked, or at least to stop entirely, until the stomach is emptied of its liquid substance.

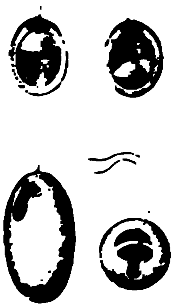
Difficulties.—The most common obstacle encountered is the clogging of the fenestra of the tube with particles of undigested food; and whenever this occurs, the tube must needs be removed and again introduced, or the funnel of the tube elevated and a few ounces of water poured into it. In case the water dislodges the obstruction, the stomach-contents and water introduced may be removed without further annoyance; but such gastric fluid is of no value for chemic analysis. Should the tube be introduced merely for the purpose of removing mucus or chemicals, this process is to be recommended.

Aspiration.—Rarely indeed will it be found necessary to employ aspiration, and for this purpose Boas' bulb-tube will be found serviceable (Fig. 131). While the stomach-tube is introduced, its proximal end is compressed and the bulb then compressed firmly; then clamp the distal end of this bulb, release the pressure at the proximal end, and permit the bulb to expand. Through this method a vacuum is created in the tube which favors the flow of gastric fluid. Another method which may be employed is to connect the tube with an aspirating bottle, such as is employed for the removal of exudates from the pleural cavities. I have not obtained satisfactory results with either of these methods, and am inclined to regard siphonic action as preferable.

Washing the Stomach.—Where the stomach is washed out merely for the purpose of removing mucus or long-retained food or for its psychic effect upon the patient, the tube should be introduced as previously described, and its funnel held at a point about four inches above the level of the patient's mouth (greater elevation may induce gastric pain and is liable to excite violent spasm of the stomach). Then the funnel should be filled with warm water and the water allowed to flow gradually into the stomach. In this manner 500 to 1000 c.c. of fluid may be introduced, but in order that this quantity be taken, the expression of the patient must be watched carefully, and upon the slightest evidence of discomfort or of retching the tube should be clamped below the funnel and the flow discontinued for a few seconds, when, in the majority of instances, the introduction of the fluid may be continued without special annoyance to the patient. In order to remove this fluid direct the patient to lean forward and drop the funnel into a vessel standing at his feet. The fluid will flow into the vessel gradually without moving the tube, and the entire stomach-contents will be emptied. This washing may be repeated two or even three times at a single introduction of the tube.

Removal of Poisons.—In attempting to remove poison from the stomach it is well to introduce as much warm water as possible before attempting to encourage the flow of the gastric contents. It is practically impossible, and always extremely difficult, to empty the stomach of its contents through the stomach-tube after a full meal; and when this is necessary, it is well first to induce vomiting either by the drinking of liquids (warm mustard water) or by the administration of apomorphin ([illegible] grain hypodermically).

Removal of the Tube.—Grasp the tube at a point about three inches distant from the patient's mouth, and compress it firmly between the thumb and index-finger; then withdraw the tube gradually—do not release the pressure until the tube is returned into the jar in which it was placed immediately before its introduction. Where no fluid has been obtained from the stomach the tip of the tube is held over a receptacle and the pressure released in the hope that a small quantity of gastric fluid may have been contained in the tube and will in this way be recovered.



[illegible]

Cleansing the Tube.—The tube may at times become plugged with a particle of food, and should it be impossible to dislodge this substance by elevating the funnel as high as possible and filling it with water, the funnel should be suddenly lowered to the other extreme, in the hope that the combined forces of siphonage and aid induced by the patient may accomplish this end. Should it be impossible to dislodge the obstruction in this manner, remove the tube and compress it firmly between the thumb and index-finger, beginning at its tip and gradually extending toward the funnel; compression and moderate stretching of the tube will usually dislodge this substance. Rarely it is necessary to dislodge the obstructing particle of food by air-pressure, for which purpose Politzer's bag will be found of service.

Einhorn's Instrument. Einhorn advocates a most ingenious device (Fig. 134) for the purpose of recovering small quantities of fluid from the stomach in persons where there exist certain counterindications to the introduction of the stomach-tube.

This instrument consists of a silver, olive-shaped vessel, eleven-sixteenths of an inch long by five-sixteenths of an inch at its greatest width. The opening of this instrument is rather large, and immediately above it there is attached a silver thread 16 inches in length. The instrument is swallowed for the whole length of the thread, and should it not pass for that distance, it is to be understood that the instrument has not yet reached the stomach. Allow the instrument to remain in the stomach for five minutes, when remove by gentle traction upon the silver thread.

By this method it is usually possible to obtain sufficient gastric fluid for the purpose of testing for free hydrochloric acid, lactic acid, and possibly for the total acidity; yet it is apparent that but a limited quantity of fluid can be obtained through the use of this instrument.

TEST-MEAL.

The secretion of gastric juice is continuous, but the quantity that we are able to obtain with the stomach-tube is usually small, except during the process of digestion, and for this reason it is preferable that the stomach-glands be stimulated to activity before attempting to collect the gastric fluid. The most efficient and practical method whereby we can accomplish this end is through the administration of a test-meal; although glandular activity of the stomach is to be accomplished by thermic and electric stimulation to this viscus. The test-meal, furthermore, gives an idea of the secretory, resorptive, motor, and digestive powers of the stomach.

The results of chemic analysis of the gastric juice will be found to fluctuate greatly, depending upon the character of food administered at the test-meal. It has been found that starches and fats stimulate gastric secretion but moderately, but that a profuse secretion follows the ingestion of proteids. The gastric fluid is influenced by the taking of fluids at the time of the test-meal, or immediately before the gastric contents is collected. Again, the height of digestion will be found to vary in time after the administration of a test-meal, this variation depending upon both the quantity and the character of the food ingested. The various investigators have endeavored, therefore, to recommend a test-meal of definite composition and volume, advising that the gastric contents be withdrawn at a certain time after the administration of this meal. I have deemed it profitable, therefore, to describe the following test-meals:

Ewald-Boas' Test-breakfast.—The test-breakfast of Ewald and Boas consists of 35 to 70 grams (1 to 2 oz.) of wheat bread and 300 to 400 c.c. (one and one-half to two glasses) of water. Weak tea without sugar may be substituted for the water. This meal should be administered early in the morning, when the stomach is empty, and before any food or liquid has been taken. The plan which I have adopted is first to wash out the stomach thoroughly, taking careful note—(1) of the quantity of gas that escapes upon the introduction of the tube; and (2) of the mucus contained in the washing. The test-meal is now given, the patient being directed to eat the bread slowly and to sip the water while eating.

Collect the gastric contents one hour after the test-meal has been ingested.

Riegel's Test-dinner.—The test-dinner of Riegel consists of 400 c.c. (13.5 fl. oz.) of soup, 200 grams (7 oz.) of beef-steak, 50 grams (1.7 oz.) of wheat bread, and 200 c.c. (one glass) of water.

Collect the stomach-contents four hours after the meal has been given.

Caution.—The steak given at this meal should be chopped finely, lest particles of meat obstruct the lumen of the stomach-tube.

Boas' Test-breakfast.—Boas' test-breakfast consists of oatmeal soup (a tea-spoonful of rolled oats in one liter of water, boiled down to 500 c.c.—17 fl. oz.) and sufficient salt added to render the soup palatable. In this meal Boas' object was to obviate the introduction of lactic acid with the test-meal, and, by avoiding bread, which contains a certain amount of lactic acid, this end is accomplished. This test-breakfast is necessary only where it is desired to estimate the quantity of lactic acid present; and where this knowledge is required, it is well to wash the stomach thoroughly the night before the administration of the test-breakfast.

Collect the gastric contents one hour after the meal has been taken.

Salzer Test-meal.—The double test-meal of Salzer consists of a breakfast of 30 grams (1 oz.) of lean, cold roast-beef, which is chopped sufficiently fine not to obstruct the lumen of the stomach-tube; milk, 250 c.c. (8 fl. oz.); rice, 50 grams (1.7 oz.); and a soft-boiled egg. Four hours later the Ewald-Boas test-breakfast is given.

Collect the gastric contents one hour later—five hours after the administration of the first meal.

Through this double test-meal the gastric juice is obtained at the height of digestion, and the stomach's power of motility is also appreciated, which motility under normal conditions is sufficient to have removed the first meal entirely. Whenever the gastric contents is found to contain particles of meat administered with the first meal, the motor power of the stomach is diminished.

CHARACTERISTIC FEATURES OF THE GASTRIC JUICE.

The gastric juice, when pure, is an almost clear, faintly yellow liquid, having a sour taste, a peculiar odor, and a specific gravity varying between 1.002 and 1.003. The reaction of the gastric juice is acid, due to the presence of hydrochloric acid, and is found to contain about 0.5 per cent. of solids.

Amount.—Bidder and Schmidt conclude that the gastric juice secreted during the twenty-four hours is equivalent to about one-tenth the body weight; while Beaumont estimates the quantity of gastric juice at 180 grams a day. There can be but little doubt that the quantity of gastric juice secreted varies widely both in health and in disease; and that such conditions as diet, exercise, general nutrition, and environment, as well as age, sex, and occupation, all influence materially the glandular activity of the stomach. Boas claims that from 20 to 50 c.c. (1 fl. oz.) should be extracted from the stomach of a healthy individual one hour after the ingestion of Ewald's test-meal; and I have always found it possible to extract this quantity of fluid under similar conditions. It is usually possible to recover from 1 to 50 c.c. of fluids from a stomach during the non-digesting period. The quantity of fluid obtained after a test-meal is governed by the motor power of the stomach; the size of the stomach; the degree of transudation; the degree of resorption; by the fluid ingested with the test-meal; the power of contraction of the stomach, and the activity of the gastric glands.

Excess.—An excessive quantity of gastric juice is to be found in connection with a hypersecretion, and may occur only at certain periods, or in rare instances this may be constant. It is impossible to establish a diagnosis of hypersecretion except where the gastric fluid is withdrawn and carefully measured at various hours during the day, at which times from 100 to 1000 c.c. of pure gastric juice are to be obtained from the so-called non-digesting stomach. It is also well to empty the stomach before retiring, and again to insert the tube in the morning after a night's rest. In this way it is possible to recover an excess of gastric fluid during the course of certain pathologic conditions; but it is hardly probable that such fluids are always true gastric juice.

MOTOR POWER OF THE STOMACH.

The motor power of the stomach should be such as would rid this viscus of all particles of food, or practically all solid particles, within a period of six hours after the administration of Riegel's test-meal. It should also be found empty in from one and one-half to one and three-fourth hours after the ingestion of Ewald's test-meal.

Clinical Significance.—The food will be retained in the stomach for an abnormally long period wherever dilatation exists, such retention varying in time, dependent upon the degree and character of the dilatation. Where the dilatation is extreme, it is possible to recover from the stomach great quantities of food, some of which may have been eaten several days before.

Food may be recovered from the stomach in from twelve to twenty-four hours after it has been taken in cases of acute gastritis, and less often is this so in subacute gastritis. The food may be retained in the stomach where a simple atony exists.

Leube's Method.—The stomach is thoroughly emptied through the stomach-tube and then Riegel's test-meal administered. Six hours later reintroduce the stomach-tube and withdraw as much as possible of the stomach-contents; then introduce about 300 c.c. (10.14 fl. oz.) of warm water through the tube; withdraw this quantity. Repeat this washing two or three times until no particles of food escape through the tube and the wash-water is clear.

Should this washing contain only minute particles of food, the motor power of the stomach is normal. This method is highly practical on account of its ease of execution, and in my experience has given most satisfactory results.

Ewald-Sievers' Method.—This method depends upon the fact that salol is broken up into phenol and salicylic acid when introduced into an alkaline medium; and that whenever this change is effected, the salicylic acid is eliminated through the urine in the form of salicyluric acid. The time at which the salol passes from the stomach (an acid medium) into the intestine (an alkaline medium) is, therefore, placarded by the appearance of salicyluric acid in the urine.

A capsule containing 1 gram (15 gr.) of salol is administered after a full meal. Collect in separate bottles a portion of the patient's urine, voided one-half hour, one hour, one and one-half, two, twelve, and twenty-four hours later. These different specimens should all be tested for the presence of salicyluric acid: Place a few cubic centimeters of the filtered urine in a test-tube, and to it add a small quantity of a solution of sesquichlorid of iron. Salicyluric acid causes a violet color with this reagent (see *Urine*, page 256).

Clinical Significance.—After the introduction of the capsule of salol into a normal stomach a positive reaction is given with the urine voided from forty-five to seventy-five minutes later, and where the reaction is retarded, the motor power of the stomach is diminished. The specimen of urine obtained at the end of the twenty-four hours should, under normal conditions, give a negative result, showing that the salol had been eliminated prior to the secretion of this specimen. Where gastric dilatation exists, the urine may give a positive result after twenty-four hours. Failure to obtain a reaction for salicyluric acid with any of the specimens collected during the twenty-four hours points to pyloric stenosis.

Fallacies.—It is to be borne in mind that salol is rarely decomposed in the stomach through the agency of alkaline mucus, which expedites the reaction for salicyluric acid. The intestinal fluid may be acid in reaction as a result of fermentative changes (acetic and butyric fermentation). This condition delays the reaction for salicyluric acid.

RESORPTIVE POWER OF THE STOMACH.

The resorptive power of the stomach is best ascertained in the following manner: Just prior to the taking of a full meal a capsule containing 0.2 gram of potassium iodid is given. The saliva is now to be tested, at intervals of from two to four minutes, for the presence of potassium iodid, by strips of filter-paper wet with stock solution. When the resorptive power of the stomach is normal, the paper takes on a violet color in from seven to eleven minutes after the ingestion of the capsule; and in from seven and one-half to fifteen minutes a blue tint may also be found.

Clinical Significance.—The resorptive power of the stomach is delayed in gastric dilatation and in carcinoma. A less marked retarding of the appearance of potassium iodid in the saliva is to be found in chronic gastritis, and, at times, where gastric ulcer exists. Exceptions to this rule are by no means uncommon.

GÜNZBURG'S METHOD FOR INDIRECTLY EXAMINING THE GASTRIC JUICE.

Günzburg suggested the following method for the indirect examination of gastric contents of persons wherein there existed some counterindication to the use of the stomach-tube: Place a tablet containing 0.2 to 0.3 gram of potassium iodid in an extremely thin but well-vulcanized piece of rubber tubing, which, when the ends of the package are folded neatly, will vary from 2 to 2½ cm. in length. Around this tubing are tightly tied two or more threads of fibrin which have been previously treated with alcohol. Günzburg has advised that each package be placed in warm water for several hours before use, and that later this water be examined for the presence of potassium iodid. In the event of no iodid being present, the package is considered fit for use. The bands of fibrin are dissolved when brought in contact with free hydrochloric acid, and their dissolution is indicated by the appearance of the potassium iodid in the saliva.

The patient is previously prepared and given Ewald's test-breakfast (p. 315); forty-five minutes later he is permitted to swallow the packet containing the potassium iodid. Examine the saliva for the presence of potassium iodid at intervals of from twelve to fifteen minutes. It may at times be necessary to continue these examinations for a period of six hours before a positive reaction for iodid will be obtained.

Clinical Significance.—When this packet is introduced into a normal stomach, the saliva should give a positive reaction for potassium iodid after a period of one to one and three-quarter hours has elapsed. When hyperchlorhydria is present, the reaction may be tardy, appearing in from two and one-half to four hours. A similar result is also witnessed when hypochlorhydria exists. When a positive reaction is not given by the saliva for a period of from five to six hours after the ingestion of the packet, anchlorhydria probably exists.

Günzburg's packets are prepared abroad, and for this reason have not come into common use in America.

CHEMISTRY OF THE GASTRIC CONTENTS.

The gastric juice consists of water, free hydrochloric acid, ferments, zymogens, and mineral acids; but an accurate analysis of the normal human gastric fluid is impossible, since this fluid is always contaminated with an admixture of saliva. It has been possible to obtain an analytic report of analyses of the gastric juice in a case suffering from gastric fistula, and, according to Schmidt, the following table will designate the solid constituents per thousand parts of gastric juice:

Water	994.4
Solids	5.6
Organic material	3.19
Sodium chlorid	1.46
Calcium chlorid	0.06
Potassium chlorid	0.55
Ammonium chlorid	not given.
Hydrochloric acid	0.2
Calcium phosphate Magnesium phosphate Iron phosphates	0.12

It is to be borne in mind that in the foregoing analysis it was impossible to exclude from the gastric juice the buccal secretions; hence the table, more correctly speaking, represents the chemic constituents of the gastric fluid when mixed with saliva.

ACIDITY.

Schmidt has satisfactorily shown that the acidity of normal gastric juice depends upon the presence of free hydrochloric acid. Experimentally it has been demonstrated that by determining the amount of chlorin and basic constituents present in the gastric juice the latter substances, after having been thoroughly saturated, still permit a quantity of hydrochloric acid to remain. In the dog this varies between 0.25 and 0.42 per cent.—average, 0.35 per cent. It is generally conceded that the acidity of pure gastric juice is dependent upon the presence of free hydrochloric acid, but when contaminated with saliva or mixed with foods in the various stages of digestion, other factors are to be taken into consideration.

After the ingestion of food, a varying amount of lactic acid, derived from the carbohydrates and acid salts, is also present; in fact, Ewald regards the gastric acidity during the early stage of digestion as depending, in a variable measure, upon the presence of lactic acid. It is to be borne in mind, however, that hydrochloric acid is also present at this stage of digestion, but it probably exists in rather close combination with albuminous substances. Whenever the albuminous substances present in the stomach have become saturated, hydrochloric acid appears in the gastric contents, and this appearance is followed by a lessening in the formation of lactic acid; the quantity of the latter gradually decreases, since hydrochloric acid inhibits the development of the micro-organisms concerned in the production of lactic acid. The degree of acidity to be noted at the different stages of digestion is illustrated by the accompanying charts, which have been designed by Rosenheim (Figs. 135, 136). In disease the quantity of free hydrochloric acid may display wide variations, and in extreme conditions it may be absent (gastric carcinoma), or even reach a maximum point of 0.5 per cent. (gastric ulcer). Again, the quantity of lactic acid which, under normal conditions, is but slight or absent during the height of digestion, is often found increased, and fatty acids are likely to be present at this time.

The total acidity of the gastric contents will, therefore, be seen to depend upon the presence of some one or more of these acids, but a high degree of acidity does not point directly to the presence of any one acid in excess, and it is, therefore, necessary to determine the presence or absence of the various acids and of acid salts in order to determine upon what particular substance present the acidity depends.

Tests for Free Acids.—1. Test the gastric contents with litmus-paper.

2. The next step is to determine whether the acidity present be due to free acids or to acid phosphates: (*a*) Dip about one inch of a slip of Congo-red test-paper into the gastric fluid, when, in the presence of free acids, this paper is turned blue—a change not induced by acid phosphates; but should combined hydrochloric acid also be present in a large amount, this, too, will cause a blue reaction.

Congo-red Test-paper.—Place one decigram of Congo red in 100 c.c. of water; cut bibulous paper in narrow strips, and soak them in this solution, from which they are to be removed and allowed to dry in the air. Place in a colored, glass-stoppered bottle.

Congo-red test-paper is less delicate than when the same reagent is employed in solution, and will only indicate the presence of 0.01 per cent. of hydrochloric acid; but by using a weak solution of Congo red, 0.0009 per cent. of hydrochloric acid may be detected. This is accomplished by adding one or more drops of the Congo-red solution to from 5 to 10 c.c. of the filtered gastric juice. In the presence of free acids a blue color develops which will be seen to vary through the successive shades from that of a sky-blue to a deep azure.

Place 5 c.c. of the gastric fluid in a test-tube and add to it a small amount of calcium carbonate. Should effervescence occur, free acid is present. Filter this fluid, and should the filtrate still retain its acidity, acid phosphates are present; free acids being neutralized by calcium carbonate, while acid phosphates are not materially influenced.

Clinical Significance.—A negative result is conclusive evidence that peptic activity is absent, since pepsin is active only in solutions that contain free acids. A positive reaction calls for further tests to determine the presence of free hydrochloric acid, lactic acid, or fatty acids.

Total Acidity.—*Reagents.*—(1) A $\frac{1}{10}$ normal solution of sodium hydrate (4 gm. in 1000 c.c. of distilled water); (2) an alcoholic solution of phenolphthalein (1 per cent.).

The total acidity of the gastric contents depends upon the following factors: Free hydrochloric acid; hydrochloric acid in combination; acid salts, and organic acids (lactic and butyric).

Estimation.—The acidity of the gastric contents is ascertained by titrating a given quantity of gastric juice with the $\frac{1}{10}$ normal solution of sodium hydrate, a few drops of the phenolphthalein solution having first been added.

Jaworski and Ewald have proposed calling the number of cubic centimeters of the sodium solution that are required to neutralize 100 c.c. of the stomach-contents the "degree of acidity," and, indeed, this method, if universally employed, would in a measure simplify computation.

Technic.—1. Place 10 c.c. of the gastric contents in a beaker.

2. Add a few drops of the phenolphthalein solution.

3. Add from a buret the $\frac{1}{10}$ normal sodium-hydrate solution (a few drops at a time), stirring the mixture with a glass rod after each addition of the soda solution.

4. At the point where the gastric contents assumes a dark flesh color its acidity has been neutralized by the sodium solution; and the number of cubic centimeters of the $\frac{1}{10}$ sodium-hydrate solution employed is read from the buret.

Let us suppose that it required 4.8 c.c. of the $\frac{1}{10}$ normal sodium-hydrate solution to neutralize 10 c.c. of the stomach-contents: the decimal point is then removed one place to the right, giving us the figure 48, which figure represents the acidity of the stomach-contents.

Normal.—Normal gastric juice will be found to give a figure between 40 and 60.

The total acidity is also expressed in terms of HCl, and is readily calculated according to the well-known laws of molecular equivalents (1000 c.c. of the $\frac{1}{10}$ normal solution contain 4 gm. of NaOH, which is equivalent to 3.65 gm. of HCl; should 50 c.c. of the $\frac{1}{10}$ normal soda solution be needed to neutralize 100 c.c. of gastric juice, $\frac{1}{20}$ of a liter of the $\frac{1}{10}$ normal soda solution was needed, or the equivalent to $\frac{1}{20}$ of 3.65 gm. of HCl). Hence the acidity of this 100 c.c. of gastric fluid is equivalent to that of 0.18 gm. of HCl; in other words, the acidity is 0.18 per cent.

Under normal conditions the acidity in terms of HCl will be found to approximate 0.2 per cent.

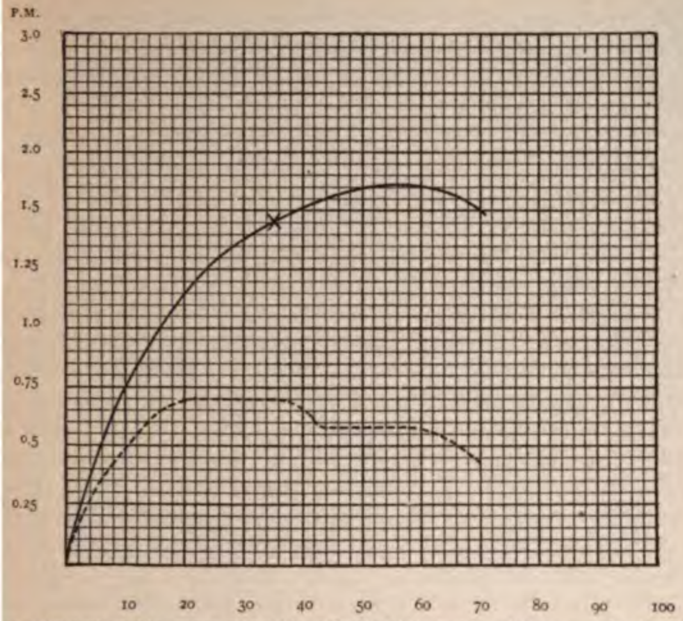
Hyperacidity.—A high figure is to be obtained in cases of gastric ulcer, gastric dilatation, gastric neurosis (less often), and where there is a hypersecretion of gastric juice.

Under pathologic conditions the acidity of the gastric juice may not depend upon hydrochloric acid alone. In fact, the total acidity may be extremely high in gastric contents devoid of hydrochloric acid, but rich in fatty and lactic acids.

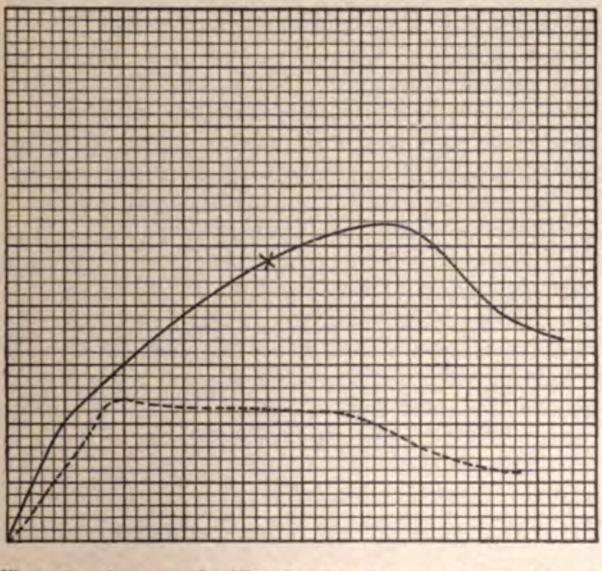
Hypoacidity.—The gastric juice may rarely be of an alkaline, neutral, or amphoteric (changes red litmus-paper to blue and blue litmus to red) reaction, but it is rather unusual to obtain alkaline gastric fluid through the stomach-tube. The acidity of the gastric juice is lowered in the mucous forms of chronic gastritis, and where there has been a complete destruction of the gastric glands. I have found the reaction of the gastric fluid neutral upon several occasions, but without exception the patient either had taken a large quantity of fluid a short time before the recovery of the gastric contents or he was suffering from a chronic catarrhal condition of the throat, and consequently swallowing an excessive quantity of buccal secretion.

When the gastric contents is collected by allowing the patient to eject it through the act of vomiting, an alkaline reaction is commonly obtained, and this alkalinity is in part, if not entirely, due to the admixture of secretions from the throat and buccal cavities.

Amount of Hydrochloric Acid.—In health, pure gastric juice has been estimated by Ewald, Boas, and Szabo to contain from 0.2 to 0.3 per cent. of free hydrochloric acid. Such quantities of hydrochloric acid are to be found only in gastric juice obtained at the height of digestion, after all the albuminous and basic affinities have been satisfied. The exact period at which this height of digestion is reached is governed by the character and the quantity of the food and liquids previously ingested: the less work there is to be accomplished, the sooner will the gastric contents be found to contain free hydrochloric acid following the ingestion of food. Following the ingestion of Ewald's test breakfast the hydrochloric acid appears in from thirty to thirty-five minutes; but the point of maximum intensity is usually reached in from fifty minutes to an hour, and may correspond to 1.7 to 0.17 per cent. (Fig. 135). After the administration of Riegel's meal the free acid appears after one hundred and thirty-five minutes, and reaches its highest point, corresponding to 0.27 per cent., in from one hundred and eighty to two hundred and ten minutes (Fig. 136).



35.—Illustrating the curve of acidity after Ewald's test-breakfast: ▬, Hydrochloric d; ---, lactic acid; ×, beginning of the stage of free hydrochloric acid (Rosenheim).



36.—Illustrating the curve of acidity after Riegel's test-meal: ▬, Hydrochloric acid; --, lactic acid; ×, beginning of the stage of free hydrochloric acid (Rosenheim).

The following terms are applied to designate the degree or proportion of hydrochloric acid secreted: *Euchlorhydria*, a secretion of the normal amount of free hydrochloric acid, varying from 0.1 to 0.2 per cent.; *hyperchlorhydria*, the secretion of more than 0.2 per cent.; *hypochlorhydria*, the secretion of less than 0.1 per cent.; and *anchlorhydria*, where no hydrochloric acid at all is secreted.

Euchlorhydria.—This condition is most frequently observed in connection with nervous dyspepsia, and is always to be found where no gastric derangement exists.

Rarely a normal secretion of free hydrochloric acid is found where atony of the muscular wall of the stomach exists. The presence of a normal amount of free hydrochloric acid tends to exclude the existence of chronic gastritis and favors the presence of the so-called nervous dyspepsia.

Hyperchlorhydria.—A hypersecretion of hydrochloric acid, as a rule, indicates gastric neurosis, and is commonly encountered in the gastric fluid of neurasthenic persons. A hypersecretion of hydrochloric acid is at times coupled with a continuous hypersecretion of gastric juice.

Hyperchlorhydria is commonly encountered in cases of gastric ulcer, and in three instances I have observed it in gastric carcinoma. Theoretically, and probably in every instance of this nature, the gastric carcinoma was preceded by, and may be developed at the site of, an old ulcer.

Hypochlorhydria.—This condition is associated with pathologic states where the secretory power of the stomach is inhibited or lessened as a result of disease; and is to be observed in connection with subacute and chronic gastritis, duodenal ulcer, gastric carcinoma, dilatation, atony, and, rarely, in cases of gastric ulcer.

Anchlorhydria.—This condition is most commonly met in gastric carcinoma, although an absence of free hydrochloric acid is not essentially to be found in this disease, as has been set forth under hyperchlorhydria. Anchlorhydria may be observed in connection with chronic gastritis and in neurasthenic individuals. It will be found that the degree of acidity of the gastric contents will fluctuate within wide limits during the course of the various chronic diseases, and especially in those placarded by cachexia and emaciation.

Source and Significance of Hydrochloric Acid.—It is not the purpose of this volume to deal with the physiology of the gastric secretion and of its one important constituent, hydrochloric acid. It will suffice, however, to state that the hydrochloric acid is not derived from the chlorids ingested; its secretion is continuous, and the total acidity of the stomach-contents in no way depends entirely upon the quantity and the quality of the food taken. The chlorids of the blood furnish the necessary chlorin to the gastric contents. A fact ever to be borne in mind is that the pyloric glands furnish an alkaline secretion, while the glands of the fundus furnish an acid secretion. It is thought that the parietal cells are concerned in the production of hydrochloric acid. A further and more elaborate description of the physiology of this secretion has been omitted because the entire subject of the production of hydrochloric acid remains undetermined. (The reader is referred to special works upon physiology and physiologic chemistry for a more complete discourse upon this subject.)

It seems clear that the factor to be concerned in the production of hydrochloric acid is a normal condition of the blood and of the secretory cells. Experience shows that whenever any decided abnormality exists in either the blood or the secretory cells, hydrochloric acid is secreted in abnormal quantities; or, in fact, there may be no secretion whatever. A consideration of serious importance is the condition of the general nervous system, since nerve energy figures largely in many forms of gastric derangement and since the cells are dependent upon this tissue for their innervation. Circulatory embarrassment of the stomach may result in impaired nutrition of the secretory cells, whether this condition be inflammatory or otherwise, and it is for this reason that the gastric secretion is materially altered during the course of inflammatory processes of the stomach, tumors, congestion, and during such chronic conditions as nephritis, hepatic cirrhosis, pulmonary tuberculosis, valvular heart disease, and, in fact, all conditions accompanied by anemia. Here may be the opportune place to make mention of the fact than an analysis of the gastric contents is of but limited value where the patient is suffering from anemia, either primary or secondary, the cause of which is not of gastric origin.

Function.—The reader is referred to special works on physiology for the functions of the hydrochloric acid. From a clinical as well as from a physiologic standpoint the antiseptic properties of the hydrochloric acid become of special importance.

It has been shown by various observers that hydrochloric acid possesses a germicidal power under certain conditions; and it has been further proved that normal gastric juice destroys the cholera bacillus, Streptococcus pyogenes, Staphylococcus pyogenes aureus, bacillus of anthrax, Shiga's bacillus, and numerous other pathogenic bacteria. It is worthy of mention that the tubercle bacillus is not destroyed by gastric juice, and that a portion of the spores of anthrax escape destruction when placed in the gastric fluid. Hydrochloric acid inhibits the development of the bacteria concerned in the production of lactic and butyric acids, although these eventually escape complete destruction, since they are always present in the gastric fluid when the degree of hydrochloric acid is at a low ebb, as has been described under *Acidity* (page 321).

Tests for Free Hydrochloric Acid.—It may prove of value to the student to know a number of reagents in the order of their delicacy, through the use of which it is possible to detect free hydrochloric acid in the filtered gastric juice:

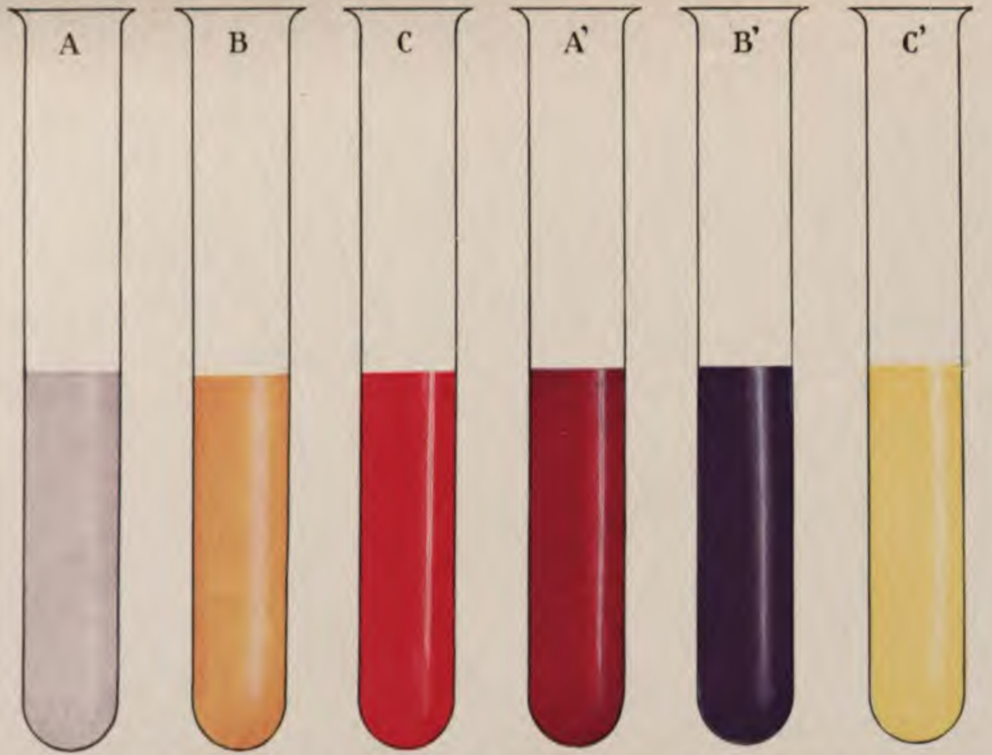
Dimethylamido-azobenzol	0.02	pro mille.
Phloroglucin-vanillin	0.05	" "
Resorcin	0.05	" "
Congo red		
Tropæolin 00	0.3	" "
Emerald-green	0.4	" "
Mohr's reagent	1.0	" "

Dimethylamido-azobenzol.—This test is extremely delicate, and its accuracy has been such that it is personally recommended most heartily to the profession. To demonstrate its delicacy the reagent is employed where a neutral yellow indicator has been added, and is changed to a reddish or cherry tint upon the addition of but one drop of a $\frac{1}{10}$ normal solution of hydrochloric acid in 5 per cent. of distilled water. A red color is also produced with organic acids, should they be present in amounts above 0.5 per cent., but this reaction is prevented whenever small quantities of mucin, peptones, or albumin are present; and since one or more of these bodies are commonly present, it is seldom, if ever, that the reaction is caused by organic acids unless a much larger amount is present. No color is produced by the loosely combined hydrochloric acid nor by the acid salts.

Method.—Reagents.—A 0.5 per cent. alcoholic solution of dimethylamido-azobenzol: (1) Place 5 to 20 c.c. of filtered gastric contents in a test-tube (it is not essential that the contents be filtered, though it is my practice to filter); (2) hold this test-tube in a good light, and add to it, from a pipet, one to three drops of dimethylamido-azobenzol solution.

If free hydrochloric acid is present, a cherry-red color is formed (Plate 22), the intensity of which varies in direct ratio to the quantity of free acid present. Should there be no free hydrochloric acid present, the fluid becomes cloudy, at times fluorescent, or may assume a yellowish tint. The reaction changes

PLATE 22.



A. Gastric fluid to which a 1 per cent. solution of phenolphthalein has been added.
B. Gastric fluid to which a 1 per cent. solution of alizarin has been added.
C. Gastric fluid to which a 0.5 per cent. solution of dimethylamido-azobenzol has been added.
A'. A after titration with a decinormal solution of sodium hydrate.
B'. B after titration with a decinormal solution of sodium hydrate.
C'. C after titration with a decinormal solution of sodium hydrate.

to a yellow when titrated with a decinormal solution of sodium hydrate (hydroxid), as shown by Plate 22, C and C′.

Phloroglucin-Vanillin Test.—*Reagents.*—Place in a mortar 2 gm. of phloroglucin and 1 gm. of vanillin and dissolve in 30 c.c. of absolute alcohol. This solution is at first of a yellow color, which gradually darkens to a golden-red, and if exposed to the light, changes to a brown. Keep in a colored glass-stoppered bottle.

It has been recommended that the two substances be kept in separate solutions, and that two or more drops of each be mixed when the reagent is to be used.

According to Boas, the above solution will be found slightly more delicate and decidedly more staple when the above proportions of the phloroglucin and vanillin are dissolved in 100 gm. of 80 per cent. alcohol.

Method.—Place a few drops, or, if desired, a few cubic centimeters, of filtered gastric contents in a dish or in a test-tube, and to it add an equal quantity of the reagent. No change occurs until the mixture is *gently heated,—avoid boiling and evaporation!*—when in the presence of 0.05 per cent. or more of free hydrochloric acid fine rose-colored lines form throughout the mixture. I prefer to carry on the reaction in a porcelain dish or cover (Plate 23), allowing the evaporation to take place slowly, when a rose color is indicative of the presence of free hydrochloric acid. The intensity of this color varies in direct proportion to the quantity of free hydrochloric acid present. Should the dish be heated at too high a temperature, a brown, brownish-yellow, reddish, or reddish-brown color is obtained; a similar result is at times obtained in the absence of free hydrochloric acid.

This reaction is not induced by the presence of organic acids, nor do they in any way interfere with the reaction; neither is it affected by albumins, peptones, or acid salts.

Test-paper.—Certain workers prefer to prepare a test-paper by soaking narrow strips of ash-free filter-paper in the test solution, and further to prepare and employ this paper as directed under the dimethylamido-azobenzol test (page 328). After the filter-paper is moistened with a drop of gastric fluid it is to be placed in a porcelain dish and heated gently. In the presence of free hydrochloric acid a rose color develops which is not altered by ether.

Resorcin Test.—*Reagent.*—Dissolve 5 gm. (7.7 gr.) of resublimed resorcin and 3 gm. (4.4 gr.) of cane-sugar in 100 gm. (3.5 oz.) of 94 per cent. alcohol. Keep in a colored glass-stoppered bottle.

Reaction.—1. Place from three to five drops of the filtered gastric juice in a porcelain dish, and to it add an equal quantity of the reagent.

2. Grasp the dish firmly in a pair of forceps, and hold it sufficiently close to the flame of a Bunsen burner to cause a slow evaporation of the liquid, continuing the heat evaporating to dryness; when in the presence of free hydrochloric acid a rose or vermilion-red mirror occupies the surface of the porcelain which was at first covered by the liquid (Plate 23).

Immediately surrounding the mirror is a zone of variable brown shade. The intensity of this color fades upon cooling.

It is also possible to prepare test-paper from this reagent, and to test the gastric contents according to the technic described in connection with the dimethylamido-azobenzol test. This test-paper, when dipped into the gastric contents, is turned a violet color, and later, upon the application of heat, changes to a brick-red, which does not change when treated with ether. The resorcin test is in no way influenced by the presence of organic acids, albumins, peptones, or by acid salts. It has been used daily in my laboratory during the past five years.

Congo-red Test.—*Reagent.*—An aqueous solution of Congo red, 1–1000.

Method.—1. Fill the test-tube to one-half its depth with water and to it add three drops of the reagent (Plate 23, B).

2. Grasp this tube between the thumb and index-finger, hold it in a clear light, and allow one or two drops of the gastric contents to fall from a pipet. Hold the tube steady to prevent all jarring. Immediately upon the drop of gastric contents coming in contact with the red solution a blue color is produced, the upper portion of the liquid changing to a pale and then to a dark blue, and, as the gastric contents traverses through the lower, red stratum of the liquid, it leaves behind a faint blue track, collecting at the bottom of the tube in the form of a light-blue sediment (Plate 23, B′). When the reaction is allowed to stand for a time, it may at first deepen, but later it is noticed to fade.

Congo red was first recommended as a test for free hydrochloric acid by von Hosslin and by Riegel in 1886, these authors suggesting that it be used in the form of test-paper. Through personal experience I have been unable to obtain satisfactory results in this manner. I have employed the Congo-red test in solution, as above outlined, during the past six years, and thus far have had no reason to change to any other test for general routine work.

Fallacies.—True, indeed, the Congo-red test is subject to certain fallacies, as are most reactions for free hydrochloric acid, and it is not to be used to the exclusion of all other tests. Personally, I employ the Congo-red and the resorcin test in every instance.

Organic Acids.—It is worthy of note that free organic acids are capable of changing Congo red to blue, but these acids are seldom, if ever, present in the gastric contents in sufficient amounts to induce this change. No color change occurs with combined hydrochloric acid, and the reaction is not influenced by albumins or by acid phosphates in such dilution as they are met with in the stomach-contents.

Tropæolin Test.—*Reagent.*—A saturated alcoholic solution of tropæolin 00. This reagent is of a brown-yellow color.

1. Place four or five drops of the reagent in a porcelain dish; grasp the dish with a forceps, and tilt it from side to side so that the reagent may flow over a large area of its surface.

2. An equal quantity of the gastric contents is now added, and in a like manner encouraged to flow over the surface of the dish.

3. Heat the dish gently over a Bunsen flame, avoiding excessive temperature; in the presence of free hydrochloric acid a lilac shade or faint blue lines appear. Boas has found this reagent to indicate the presence of free hydrochloric acid where this substance exists in from 0.2 to 0.3 per cent. For practical work this test will be found satisfactory, but in instances where a negative result is obtained, the more delicate reactions previously described are to be employed.

A test-paper may be prepared from this reagent, and when saturated with the gastric contents and gently heated, changes to the characteristic lilac or blue. In the presence of organic acids a brown color develops, but this disappears upon the application of heat; and at no time during the reaction is a lilac or blue color caused by organic acids.

Mohr's Test.—*Reagent.*—Place 2 c.c. of a 10 per cent. solution of potassium sulphocyanid and 0.5 c.c. of a neutral solution of ferric acetate in a graduate, and to the mixture add 10 c.c. of distilled water. This reagent is of a ruby-red color.

Reaction.—Place two to five drops of the reagent on a porcelain dish, and to it add an equal quantity of the filtered gastric contents. Place the reagent and the stomach-contents on different sides of the dish and permit them gradually to come in contact.

Free hydrochloric acid induces a light violet color which is deepest at the line of contact of the reagent and the gastric fluid. When the fluid and reagent have been well mixed, a deep mahogany color is seen. Mohr's test cannot be regarded as a highly sensitive one, and while it is not influenced by the presence of acid salts or of peptones, it should not be employed to the exclusion of other tests.

For a description of Cipollino's test see p. 533.

Quantitative Estimation of Hydrochloric Acid.—Töpfer's Method.—Töpfer's method for the estimation of hydrochloric acid in the gastric contents will be found sufficiently accurate for practical laboratory studies, and has to recommend it the important feature that analysis by this method is easy of execution.

Reagents.—(1) A decinormal solution of sodium hydroxid (see page 323). (2) A solution of phenolphthalein (1 per cent.). (3) An aqueous solution of alizarin (1 per cent.). (4) An alcoholic solution of dimethylamido-azobenzol (0.5 per cent.).

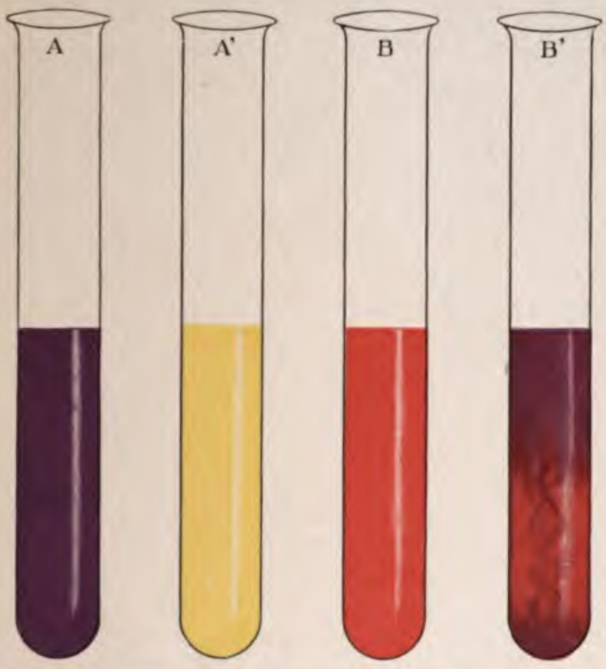
The first step in this technic is to estimate the total acidity, according to the method previously described (page 323), employing a given amount of gastric juice. This total acidity represents that due to free hydrochloric acid, combined hydrochloric acid, organic acids, and the acid salts present. Where lactic acid and the fatty acids are found to be present in the given gastric contents, it is not essential that these be removed before estimating the hydrochloric acid. Alizarin or monosulphonate of soda is also employed as an indicator, since it reacts when in an alkaline medium. The third step in Töpfer's test is to estimate the quantity of free hydrochloric acid, which is done by using dimethylamido-azobenzol as an indicator.

Method.—Place from 5 to 10 c.c. of filtered gastric juice into each of three small beakers, or, preferably, into three test-tubes (Plate 22).

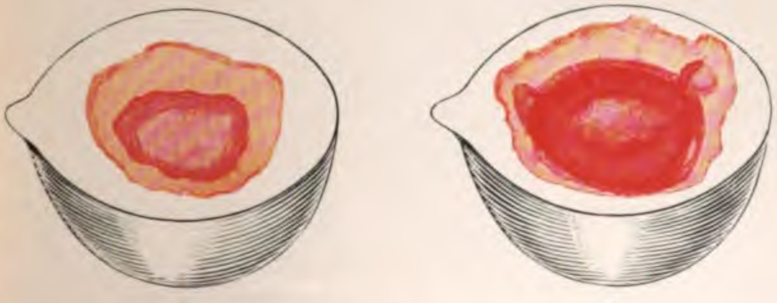
1. To the first of these tubes add two drops of phenolphthalein (Plate 22, A), when the fluid will be seen to take a light-grayish hue. Titrate the solution in tube A with a $\frac{1}{10}$ normal solution of sodium hydrate until a pink color appears, at which point the $\frac{1}{10}$ normal solution should be added, a few drops at a time, until a permanent red color is obtained (Plate 22, A′). The process of titration must be continued until the red color does not disappear when the tube is allowed to stand for a time and its contents does not become intensified upon the addition of a few drops of the sodium solution (end-reaction). Read from the buret the number of cubic centimeters of the $\frac{1}{10}$ normal sodium solution that were required to neutralize the known quantity of gastric contents.

2. Add four drops of alizarin solution to the second tube, when the contents of the tube will assume a yellowish hue (Plate 22, B). Titrate this mixture with a $\frac{1}{10}$ normal solution of sodium hydrate until a decided violet color is obtained (Plate 22, B)′.

PLATE 23.



A. Uffelmann's reagent.
A'. A after the addition of gastric fluid containing lactic acid.
B. Water to which three drops of Congo-red solution have been added.
B'. Change induced in B when gastric fluid containing free hydrochloric acid is added.



1. Resorcin test for free hydrochloric acid.
2. Günzburg's test for hydrochloric acid.

With the aid of the accompanying illustrations (Plate 22, B, B′) there will be little or no difficulty for the inexperienced to follow closely this technic.

3. To the gastric contents in the third tube add three or four drops of dimethylamido-azobenzol, when a red or rose hue is produced (Plate 22, C). Should there be no free hydrochloric acid present in the gastric contents, a yellow instead of a red color follows the addition of the dimethylamido-azobenzol solution. Titrate this red mixture with a $\frac{1}{10}$ normal solution of sodium hydrate until all the red color has disappeared and the liquid changes to yellow (Plate 22, C′).

Computation.—Let us suppose that 10 c.c. of gastric contents are placed in each of the three test-tubes. In the first 10 c.c., phenolphthalein being used as an indicator, it required 10 c.c. of the $\frac{1}{10}$ normal solution to effect the terminal or end-reaction (permanent red); but an equal quantity of gastric contents to which alizarin had been added as an indicator required only 7 c.c. to induce the end-reaction. It will be readily seen that the difference between the quantity of the $\frac{1}{10}$ normal solution employed to neutralize the 10 c.c. of gastric contents containing phenolphthalein and that needed to neutralize the 10 c.c. containing alizarin equals 3 c.c. This quantity of the $\frac{1}{10}$ normal solution (3 c.c.) indicates the amount required to neutralize the hydrochloric acid which was in combination with albuminous substances.

One cubic centimeter of the $\frac{1}{10}$ normal solution corresponds to 0.00365 gm. of hydrochloric acid; hence the amount of hydrochloric acid combined with albuminous substances present in the 10 c.c. of gastric contents examined is estimated by multiplying 0.00365 by 3, which gives 0.01095 gm. of hydrochloric acid; and this figure is also employed as 0.01095 per cent.

Combined Hydrochloric Acid.—The total acidity of the gastric contents depends in a measure upon the presence of loosely combined hydrochloric acid, and since this hydrochloric acid is essential to digestion, it is of great importance to ascertain whether or not combined hydrochloric acid be present in a given gastric contents, even where free hydrochloric acid be absent. The presence of free hydrochloric acid in normal quantities implies that peptic digestion is taking place, and, further, indicates that all the albuminous affinities present in the gastric contents have been satisfied, free hydrochloric acid appearing as such after all albuminous substances have been saturated. Free hydrochloric acid may be absent and still there may have been a certain quantity of hydrochloric acid secreted, and this acid be in combination with albuminous substances, the amount secreted being only sufficient to satisfy a portion, or possibly all, of the albuminous affinities, in which case peptic digestion, which is active only in the presence of free hydrochloric acid, does not progress.

It is readily seen, therefore, that a knowledge of the combined hydrochloric acid is of great importance in connection with most gastric disorders, particularly those wherein free hydrochloric acid is absent from the gastric contents.

An entirely different condition exists where both free and combined hydrochloric acid is absent from the gastric contents, and implies that no hydrochloric acid is secreted. Where this condition exists, the stomach acts merely as a storehouse for proteids.

Free Hydrochloric Acid.—The estimation of the free hydrochloric acid is made for 10 c.c. of the gastric contents, using dimethylamido-azobenzol as an indicator. Let us suppose that 3.5 c.c. of the $\frac{1}{10}$ normal solution were required to produce the end reaction, then $0.00365 \times 3.5 = 0.011675$, or 0.011675 per cent.

It will be remembered that in ascertaining the total acidity of 10 c.c. of gastric contents 10 c.c. of a $\frac{1}{10}$ normal sodium-hydrate solution were required, which, in terms of hydrochloric acid, are equivalent to $10 \times 0.00365 = 0.0365$ gm. of hydrochloric acid, or 0.0365 per cent. Deduct from this number the sum of the amount of both free and combined hydrochloric acid, $0.01095 + 0.012575 = 0.023725$; $0.0365 - 0.023725$, or 0.012775 per cent., which equals the percentage of acidity due to organic (lactic and butyric) acids.

Clinical Significance.—In selecting a diet for an individual where the hydrochloric acid is known to be absent it is necessary to direct that proteids be given in such form as will permit them to be subject to pancreatic digestion, obviating all possible delay. It is in such conditions that the administration of predigested foods is indicated.

At times it is found that the quantity of hydrochloric acid secreted is sufficient to satisfy the albuminous affinities of a moderate-sized meal in which the proteids have been limited, and in such cases, when a suitable dietary be followed, digestion may go on perfectly. It is also possible to give a favorable prognosis where this amount of hydrochloric acid is secreted; but where there is an absence of loosely combined hydrochloric acid, this finding points to a destruction of the glandular elements of the stomach, in which instance prognosis must, of necessity, be grave.

Tests for combined hydrochloric acid have been described in connection with Töpfer's test for free hydrochloric acid (page 332).

FERMENTS AND THEIR ZYMOGENS.

It is generally conceded that pepsin itself is not secreted as a product of the chief cells of the glands of the fundus, but that the zymogen of pepsin (pepsinogen or propepsin) is secreted. Numerous experiments have proved this statement, and shown that in fasting animals the glands of the stomach do not contain pepsin, but a substance which is not destroyed by sodium carbonate and which is readily converted into pepsin when brought in contact with hydrochloric acid. It is this substance which has been designated under the caption pepsinogen.

Pepsin is to be recovered from the mucous membrane of the stomach during the stage of digestion, but during the non-digestive stage, zymogen is to be recovered. Zymogen may be found coëxistent with pepsin in the normal gastric juice recovered during the process of digestion. The time at which zymogen is transformed into a ferment is doubtful. A fair amount of evidence exists, however, to show that this change takes place after it has been secreted.

Destruction.—Pepsin and the activity of its ferment are destroyed by dilute solutions of alkaline carbonates or exposure of a solution of pepsin to 70° C. (158° F.); but pepsin in the dry state is not destroyed by a temperature of 100° C. (212° F.).

It is to be borne in mind that albuminous substances may be digested by pepsin when in the presence of hydrochloric acid and certain other acids, as sulphuric, acetic, phosphoric, salicylic, and lactic. A solution of any one of these acids must needs be considerably stronger than is needed when the acidity is due to hydrochloric acid. Hydrochloric acid requires from two to four pro mille to obtain satisfactory results, but should we employ lactic, 12 to 18 pro mille would be necessary to accomplish the same end. In this connection the experiments of Petit are of special importance, since this observer found that a preparation of pepsin would digest 500,000 times its weight of fibrin in a period of about seven hours. It is again important that extremely small quantities of pepsin are always capable of digesting large quantities of albuminous substances.

The quantity of pepsin or of its zymogen secreted during the twenty-four hours can only be approximated from the apparent peptic activity, which is estimated through the amount of albuminous substances digested; and it is to be remembered that this will be found to fluctuate within wide limits under normal conditions, depending in a great measure upon the degree of concentration of the free acid present.

Clinical Significance.—While the study of free hydrochloric acid and that of pepsin have been considered side by side in this volume, it is to be emphasized that the recognition of pepsin in relative amounts concerns us more than does that of free hydrochloric acid. It has been stated that pepsin is formed from pepsinogen when in the presence of free acid. Therefore the absence of organic acids in appreciable quantities is conclusive evidence that free hydrochloric acid is present and that peptic digestion is active. On the other hand, when free hydrochloric acid is found, pepsin, as a rule, is present, though the reverse condition may exist. When hydrochloric acid is added to the gastric contents and the fluid is then found incapable of digesting albumins, both pepsin and its zymogen are absent; but should the zymogen be present, digestion occurs after the addition of an acid. In rare instances the gastric juice is found to be capable of digesting albumins without the admixture of hydrochloric acid, such digestion depending upon the presence of pancreatic secretion.

Impaired gastric circulation and nervous conditions of the stomach affect but slightly, if at all, the production of pepsin; and a diminution or an absence of pepsin or its zymogen is referable to a pathologic condition of the gastric glands. These features are most valuable in distinguishing between gastric neurosis, chronic gastritis, and hyperemia of the gastric mucosa, the result of circulatory embarrassment.

Test for Pepsin and Pepsinogen.—Test a portion of the filtered gastric contents for the presence of free hydrochloric acid. Should this be present, proceed as follows for the detection of pepsin and zymogen:

1. Place 25 c.c. of the filtered gastric contents in a beaker, and to it add 0.05 to 0.06 gm. of egg-albumen.
2. Place in an incubator, at a temperature of 37° to 40° C. (98.6° to 104° F.), for a period of three hours.

Under normal conditions this quantity of gastric juice thus environed will have digested the egg-albumen. Fibrin and serum-albumin may be added instead of the egg-albumen, the former being digested in one and one-half hours, the latter, in one hour.

Zymogen.—Should the gastric contents not contain free hydrochloric acid, giving negative results with the resorcin and Congo-red tests, proceed in the following manner:

1. Place 25 c.c. of filtered gastric contents in a beaker, and to it add from three to five drops of an official solution of hydrochloric acid.
2. Add the same quantity of egg-albumen, fibrin, or serum-

albumin that was added when hydrochloric acid was present (0.05 to 0.06 gm.).

3. Place in an incubator at a temperature of 37° to 40° C. (98.6° to 104° F.), as described under the test for pepsin. Zymogen (pepsinogen) will, as a rule, be found.

Quantitative Estimation of Pepsin.—Thus far I am not aware of any accurate method by which the amount of pepsin may be estimated, and the method of Hammerschlag, herein outlined, is capable of ascertaining merely relative values.

Place three Esbach tubes in a tube-rack, and back of these tubes insert a piece of white cardboard upon which the figure corresponding to each tube is marked, 1, 2, 3. Rosenburger's tube-rack will serve well for this purpose.

Tube 1.—Place in this tube a mixture of a one per cent. solution of serum-albumin in 4 per cent. of hydrochloric acid, filling to the mark U; then add 5 c.c. of filtered gastric juice, and place in the rack.

Tube 2.—Fill this tube with the serum solution to the mark U, and add to it 0.5 gm. of pepsin.

Tube 3.—Fill with the serum solution to the mark U, and add 5 c.c. (1 fl. oz.) of water.

Place the rack and its contained tubes at a temperature of 37° C. (98.6° F.) for one hour, then remove, and to each tube add Esbach's reagent (see page 211), filling to the mark R.

After the addition of the reagent place the rubber stopper in position, grasp the extremities of the tube between the thumb and index-finger of each hand, and invert the tube slowly several times in order to effect a perfect mingling of the reagent through the mixture. Replace the tubes in the rack in their respective pockets, and allow it to stand at a temperature of 18° to 20° C. (64.4° to 68° F.) for twenty-four hours. The amount of precipitated albumin is read from each tube, and that of tube 1 and 3 are compared by the reading given by tube 2, to which 0.5 gm. of pepsin was added, and which serves as the standard.

Absence of Pepsin.—Jaworski has suggested a method wherein it is possible to ascertain whether the gastric contents be absolutely free from pepsin, the enzyme, and its zymogen: Introduce into the empty stomach, through a tube, 200 c.c. of a decinormal solution of hydrochloric acid, and permit this solution to remain for one hour. Again insert the tube, and extract the stomach-contents. Should this fluid be found to be free from pepsin, it is indicative that both the enzyme and its zymogen are absent.

Test for Pepsinogen.—Boas has detailed a method of determining the relative proportion of pepsinogen present in the gastric contents.

1. Place in a number of test-tubes a mixture of gastric contents and distilled water, diluting the gastric fluid at different strengths in these tubes—*e. g.*, place in tube 1 a dilution of 1-5; tube 2, 1-10; tube 3, 1-20; tube 4, 1-30, etc.

2. Place these tubes in a rack, and number as described under the estimation of pepsin (page 337).

3. Add to each tube a definite amount of egg-albumen.

4. Place in an incubator at a temperature of 37° to 40° C. (98.6° to 104° F.).

Note carefully which of the fluids is of sufficient strength to digest the albumin—the one of greatest dilution capable of accomplishing this end represents the amount of pepsinogen present; the greater the degree of dilution, the more plentiful the pepsinogen.

Chymosin.—The profession is indebted to Hammarsten for the original information concerning the milk-curdling ferment and its zymogen (chymosin and chymosinogen). At present, Boas probably deserves credit for having brought forth most of the valuable information concerning this ferment and its proenzyme. The principal function of these substances appears to be exercised upon milk, or upon solutions containing a variable amount of casein, when rendered alkaline by preparations of lime. Casein is coagulated when in a feebly acid, alkaline, or neutral solution.

The proenzyme is supposed to be formed by the cells of the gastric mucous tissue, though a neutral aqueous extract of the gastric mucous membrane is not likely to contain this ferment, but will usually contain its zymogen. The addition of free hydrochloric acid is followed by the appearance of the ferment.

An active solution of chymosin when in the presence of free hydrochloric acid (3 pro mille) is found to be active when kept at a temperature of from 37° to 40° C. (98.6° to 104° F.); but pepsin, when thus environed, does not induce destruction. Again, zymogen is converted into an active ferment when the solution containing it is alkalinized by a solution of calcium chlorid, thus showing that in the presence of such salt free hydrochloric acid is not essential to this transformation.

Both chymosin and its zymogen are physiologically present in the gastric secretions, thus rendering an accurate knowledge of the proportionate amounts of these bodies present of clinical value. The detection of chymosin in the vomit serves as one of the valuable tests whereupon alkaline vomit is determined to be of gastric origin.

Qualitative Tests for Chymosin.—1. Place 5 to 10 c.c. of milk in a test-tube or into a beaker, and treat with from three to five drops of filtered gastric juice.

2. Place in an incubator at a temperature of from 37° to 40° C. (98.6° to 104° F.) for fifteen minutes.

In the event of coagulation having taken place at the end of this period, the enzyme is present.

Tests for Chymosinogen.—1. Place 10 c.c. of filtered gastric juice in a test-tube.

2. Render the gastric contents feebly alkaline by adding two or more cubic centimeters of a solution of calcium chlorid (1 per cent.).

3. Place the mixture at a temperature of 37° to 40° C., when, should zymogen be present, a thick, caseous coagulum forms within a few minutes.

Quantitative Estimation of Chymosin.—I am not aware of any method by which it is possible to determine, with any degree of accuracy, the quantity of enzyme present in a given gastric contents; and it is only possible, through the method here given, to ascertain merely relative values. Normal gastric juice will be found to give a reaction for the enzyme when diluted in the proportion of 1–30 or even 1–40.

1. Place in each of three to six test-tubes a definite quantity of gastric juice.

2. Place these tubes in a rack, and label A, B, C, D, E, F. Water is now added to each of the tubes, in such proportions as will effect in tube A a dilution of 1–5; B, 1–10; C, 1–15; and so on, until we reach a dilution of 1–30, the minimum dilution at which the enzyme should be active.

3. Add to each of the tubes a definite quantity of neutral milk.

4. Place the tubes at a temperature of from 37° to 40° C. (98.6° to 104° F.), and observe carefully the point of greatest dilution at which coagulation takes place.

At times the milk added may be found of an amphoteric reaction—*turns red litmus-paper blue and blue litmus-paper red.* Should coagulation occur at a dilution of 1–30, the enzyme is present in the minimum normal amount.

Quantitative Estimation of Chymosinogen.—1. In estimating the quantity of the zymogen present, tubes are arranged and labeled as described for the estimation of enzyme.

2. Place in each of the tubes an equal quantity of milk.

3. Add the feebly alkaline gastric juice to the various tubes in different quantities, producing a different dilution in each tube.

This process is further conducted in the manner described for the estimation of the enzyme, allowance being made for the quantity of the alkaline solution needed to alkalinize the gastric juice. Under normal conditions a dilution of from 1–100 or even 1–150 will be found to give positive results.

Clinical Significance.—Zymogen, when noted to be present in the gastric contents at several examinations after the administration of Ewald's test-meal, is valuable evidence and serves to exclude the existence of organic disease of the stomach, pointing rather directly, as it does, to gastric hyperemia, neurosis, etc., conditions referable to disease outside the stomach. Where the zymogen is found to be reduced to one-half that of the normal, gastritis probably exists. The less marked the reduction in the zymogen, the more favorable the prognosis. A decided reduction or an absence of the zymogen points rather strongly to a severe grade of gastritis, which may be accompanied by carcinoma, or decided degenerative changes in the gastric, secretory, or glandular tissues.

Chymosin may be present in appreciable or even in nearly normal amount in specimens where free hydrochloric acid is absent; and it is thought that a step, at least, has been achieved in the direction of a cure should free hydrochloric acid appear in the gastric contents as the result of treatment.

PRODUCTS OF GASTRIC DIGESTION.

Proteid Digestion.—According to Nothnagel's "Encyclopedia," the "determination of the different stages of hydration of albumin is so far of no practical value. In the first place, the method for determining the different intermediary stages in the digestion of albumin is too complicated for practical purposes; on the other hand, an exact knowledge of these stages is of no value to diagnosis. The value of the biuret reaction is certainly overestimated." The biuret reaction is a color-test, and is obtained by adding to the gastric contents an excess of potassium hydroxid and treating the mixture, while boiling, with a few drops of a 1 per cent. solution of copper sulphate. In the event of peptic digestion having taken place, a peculiar pink or rose-reddish color appears (see *Biuret Test*, page 217). A positive biuret reaction indicates that peptic digestion has taken place, but does not give us any information as regards the degree of the digestion of the albumins. According to Riegel, any gastric contents will give a more or less typical biuret reaction, but the intensity of this result does not in any way inform us as to the strength of the gastric juice.

Albuminoids.—Among the albuminoids which may be taken into the stomach but two of these bodies undergo gastric digestion (collagen and elastin), and through this process gelatoses and elastoses are formed, while keratin escapes digestion. Collagen and elastin do not contribute to the formation of heteroproteoses, but protoproteoses and, in turn, deuteroproteoses result, from which peptone is eventually obtained.

Digestion of Carbohydrates.—The gastric secretion is in itself unable to digest carbohydrates; but there is evidence suggestive of the fact that a certain amount of starch is transformed into sugar early during gastric digestion. This transformation is dependent upon the action of ptyalin, secreted with the saliva and taken into the stomach during the ingestion of food. The action of ptyalin continues until at least 0.01 per cent. of either free or combined hydrochloric acid has been secreted. It is to be borne in mind that a small quantity of acid inhibits this process, since the transformation of starches into sugar is most active in neutral or mildly alkaline solutions (see *Saliva*, page 453). The ferment of saliva first converts starch into soluble starch, then into erythrodextrin, achroödextrin, and eventually into maltose. Where the secretion of hydrochloric acid is abnormally great or is continuous, the digestion of starch in the stomach is soon arrested, and although a slight digestion of starch may occur, the end-products of saccharification are not formed—only the intermediary products are to be found. On the other hand, where the secretion of hydrochloric acid is deficient or absent, a reverse condition is observed.

All-important in analyses for the degree of digestion of starches is it to determine whether the end-products of starch digestion have been produced, and whether any of the intermediary products have been generated and are now present, thus indicating the degree at which the process of starch digestion was arrested.

Tests.—The intermediary stages of carbohydrate digestion are detected in the following manner:

Place 10 c.c. of the filtered gastric contents in a test-tube and to it add a few drops of Lugol's solution (iodin, 0.1 part; iodid of potassium, 0.2 part; distilled water, 200 parts). With this solution starch causes a blue color to appear; erythrodextrin, a violet or mahogany brown. Should no color change develop upon the addition of Lugol's solution, achroödextrin, dextrose, and maltose are present. Soluble starch causes the formation of a blackish-blue precipitate with Lugol's solution.

Achroödextrin possesses a greater affinity for iodin than for any other of the intermediary products. It is well to add Lugol's solution freely in order that some of the intermediary products that require large quantities of iodin for the production of the color may not escape notice.

Both maltose and dextrose react with Fehling's solution and are also capable of fermentation. Dextrose reduces Barford's reagent: Place a few cubic centimeters of the reagent in a test-tube; boil the upper stratum of the liquid, and add to it from a pipet a small quantity of the filtered gastric contents. In the presence of dextrose a precipitate of red cuprous oxid is formed. *Barford's reagent* is composed of a 1 per cent. solution of acetic acid to which a 0.5 to 2 per cent. solution of copper acetate has been added.

Clinical Significance.—In the event of carbohydrate digestion having been normal in both the mouth and the stomach, no color appears upon the addition of the Lugol's solution to the gastric contents. The appearance of a violet-blue color upon the addition of this reagent signifies that saccharification has been incomplete; and it is then necessary to examine further in order to determine whether this deficient digestion of carbohydrates be due to a deficiency of salivary ferment, to a hypersecretion of hydrochloric acid, or, as is rarely seen, to extraneous causes. It is common to find the salivary ferment deficient.

Digestion of Fat.—It has been demonstrated by Marcet's experiments that fats undergo some changes while in the stomach —splitting into glycerin and fatty acids; and it was further shown by Ogata and others that the stomach is capable of causing a slight disintegration of neutral fats. Again, that from 1 to 2 per cent. of oil is decomposed in the normal stomach within a period of two hours; and should the oil be allowed to remain for a longer period, due to distention of the stomach the result of dilatation, a large percentage of oleic acid may be found present, reaching a maximum of 6 per cent. The fat-splitting power of the stomach is relatively slight, and Riegel's students found that the fat-splitting ferment secreted by the stomach is active only upon fats that are well emulsified. This ferment is thought to be secreted by the mucosæ of the fundus.

FATTY ACIDS.

Boas and other observers have shown that the formation of fatty acids is closely connected with that of lactic acid. Butyric acid is at times derived from the lactic acid (Flügge). This change probably depends upon the fact that many, and possibly all, the micro-organisms concerned in the production of butyric-acid fermentation belong to the class of anaërobes. In this connection it is well to remember that both the Oïdium lactis and the Bacillus acidi lactici develop in the presence of oxygen.

Acetic fermentation may occur in the stomach—a process supposed to take place in the presence of alcoholic principles. This alcohol may not have been introduced into the stomach as such, but be the result of acetic-acid fermentation; or, more correctly speaking, transformed through the action of the saccharomyces (yeast) upon the sugars ingested.

Test for Butyric Acid.—Whenever the gastric contents is found to contain a large amount of butyric acid, it will emit an odor of rancid butter.

Extract 10 c.c. of the gastric fluid with 50 c.c. of ether; evaporate to dryness, and collect the residue with a few cubic centimeters of water. Add a trace of calcium chlorid, and should butyric acid be present, it will be precipitated in the form of small, oil-like globules. This precipitation, consisting apparently of oil, will be found to emit a decided odor.

Test for Acetic Acid.—Extract 10 c.c. of the filtered gastric juice with ether, evaporate, and dissolve the residue in a small quantity of water. Neutralize cautiously with a weak solution of sodium hydrate. In the presence of acetic acid sodium acetate is formed. Add to the mixture a few drops (1 to 3) of a weak solution of perchlorid of iron: acetic acid causes a dark-red color. A weak solution of nitrate of silver will also be found to cause a rather dense precipitate, but this is readily dissolved upon the addition of hot water.

Quantitative Estimation of Fatty Acids.—The Cahn-Mehring method, as modified by McNaught, is as follows:

1. Ascertain the total acidity of 10 c.c. of the gastric fluid (see page 323).

2. Place 10 c.c. of the gastric fluid in a beaker or a dish, and evaporate over a water-bath to the consistence of syrup; dilute with water and subject to titration, as in estimating the total acidity.

Deduct the figure obtained for acidity of the second 10 c.c. from that of the total acidity for the first 10 c.c. of filtered gastric contents. The difference is equivalent to the amount of fatty acids contained in the 10 c.c. of gastric fluid examined.

Estimation of Organic Acids (Quantitative).—The method of Hehner-Seemann depends upon the fact that a quantity of a decinormal sodium-hydrate solution, when added to organic acids, mixed, evaporated, and incinerated, permits the organic acids to be given off as carbon dioxid. Their alkali remains as a carbonate. The amount of carbonate is ascertained by titration with a $\frac{1}{10}$ normal solution of hydrochloric acid.

1. Place 20 c.c. of filtered gastric juice in a beaker and titrate with a $\frac{1}{10}$ normal solution of sodium hydrate.
2. Evaporate to dryness and incinerate. Do not continue heating after the ash fails to display a radiant flame.
3. Collect the residue by taking it up with water, then titrate with a $\frac{1}{10}$ normal solution of hydrochloric acid.

Read from the buret the number of cubic centimeters of the hydrochloric-acid solution necessary to neutralize the mixture, multiply by 0.00365, and the product will indicate, in terms of hydrochloric acid, the amount of fatty acids present in the 20 c.c. of gastric juice examined.

Clinical Significance.—Under physiologic conditions fatty acids are not present in the gastric fluid except where abnormally large quantities of milk and carbohydrates have been taken. Fatty acids are not to be found in normal gastric juice obtained after the administration of Boas' test-meal; nor are they likely to be found present where there exists chronic gastritis or moderate dilatation. Fatty acids are to be observed in connection with gastric carcinoma. Rarely, butyric acid may be formed in the mouth, but from this source the gastric contents seldom displays more than a trace of this acid. Again, the gastric contents may give definite reactions for butyric acid after large quantities of butter or other fats have been ingested. Acetic acid is a rather rare finding in the gastric contents, but when present and not due to the imbibition of alcohol, its significance is practically that of butyric acid.

LACTIC ACID.

It is generally conceded that the lactic acid found in the stomach is the result of bacterial development, such bacteria being normally present in the buccal secretions. Again, the gastric contents may be found to contain lactic acid during health, but in such instances the lactic acid has been introduced into the stomach with the food, as was described under *Test-meals* (page 315). The bacteria capable of forming lactic acid from carbohydrates and from sugar are omnipresent in the buccal secretion and gastric contents, and induce acid fermentation whenever sugar-bearing substances are ingested.

Granting that a certain amount of lactic acid may be introduced with the normal meal or that sugar-charged foods have been taken, it is possible for us to find lactic acid in normal gastric contents early during the course of digestion. By way of repetition, let it be borne in mind that lactic-acid fermentation is inhibited by the secretion of hydrochloric acid, and that this inhibition is perceptible even where the amount of hydrochloric acid present in the gastric contents is very small. The presence of 0.7 to 1.5 pro mille of hydrochloric acid, either free or combined, arrests the process of the lactic-acid fermentation. It is through this physiologic secretion of hydrochloric acid that lactic-acid production is discontinued during the latter stage and height of digestion; and it is authentically stated that lactic acid does not appear in appreciable quantities during the process of normal digestion.

Lactic acid, even when introduced into the stomach as such, may be absent during the latter stage of digestion, and where the gastric contents is shown to contain lactic acid early during digestion, its disappearance during the latter stage is doubtless due to a resorption of the lactic acid ingested. Thus, failure to obtain a reaction for lactic acid under such conditions has been attributed to the presence of free hydrochloric acid, which possibly interferes with the reaction.

Test for Lactic Acid.—Whenever it is desired to ascertain the quantity of lactic acid, it is necessary, first, to introduce the stomach-tube and to wash the stomach thoroughly, employing from eight to twelve ounces of water; withdraw this water, and in a like manner repeat the process several times at a single introduction of the tube (page 313). In this way any of the lactic acid that might be present as a result of stagnant material—a feature of gastric dilatation—is removed. Boas' test-meal (page 316) is now administered.

Should there be evidence of stagnation, it may be well to cleanse the stomach, as just outlined, in the evening when the test-meal is given. Collect the stomach-contents the following morning, before any food or water has been taken.

Caution.—Do not attempt to estimate the lactic acid of the gastric contents unless the stomach has been thoroughly washed prior to the administration of Boas' test-meal.

Uffelmann's Test.—*Reagents.*—(*a*) Saturated aqueous solution of the sesquichlorid of iron. (*b*) Concentrated solution of pure carbolic acid.

1. Place 10 c.c. of water in a test-tube, and to it add three drops of solution (*a*) and three drops of solution (*b*); shake the tube gently, when the mixture assumes a bluish-black color.

2. Add water, shaking thoroughly, until an amethyst-blue color results (Plate 23).

3. Grasp the bottom of the tube between the thumb and index-finger, hold in a clear light, and add a small quantity of filtered

gastric contents. In the presence of lactic acid the upper stratum of the liquid, to which the stomach-contents is added, changes to a canary or lemon-yellow color (Plate 23).

Fallacies.—It is possible to obtain a reaction for lactic acid with Uffelmann's reagent when there is present in the gastric contents an abundance of the butyric acid, acid phosphates, glucose, and alcohol. Again, lactic acid, when present, does not always give a characteristic reaction—the blue changing to gray, white, brown, or a dirty yellow, a feature not uncommon where the gastric contents is rich in free hydrochloric acid. In fact, hydrochloric acid is said to prevent the reaction. These difficulties are overcome by extracting the gastric contents with ether, through which method the presence of 0.001 per cent. of lactic acid is detected.

Place 5 c.c. of filtered gastric fluid in a glass-stoppered separating funnel and to it add 50 c.c. of neutral sulphuric ether, and allow it to stand for one-half hour. Then evaporate over a water-bath. Take up the residue with 5 c.c. of distilled water, and add this mixture to the amethyst-blue solution.

Kelling's Test.—Place 1 c.c. of the filtered gastric contents in a test-tube and dilute with 9 c.c. of water. The mixture is now held in a clear light, and one to three drops of a 5 per cent. aqueous solution of the sesquichlorid of iron are added, one drop at a time. Lactic acid causes a greenish-yellow color to appear upon the addition of the reagent, a feature characteristic of lactic acid.

Boas' Reaction.—Aldehyd is commonly met with in gastric fluid, and sarcinæ are often found. Tersely, Boas' reaction for lactic acid is to recover acetic aldehyd, a substance detectable through the employment of Nessler's solution, which is made as follows: Dissolve 2 gm. of potassium iodid in 50 c.c. of water, and add a variable amount of iodid of mercury, heating until only a portion of the mercury remains undissolved. Upon cooling, add to the mixture 20 c.c. of water, and place in a glass-stoppered bottle. Two parts of this solution are to be added to three parts of concentrated potassium hydrate. After the mixture is allowed to stand for some time, filter and place in a glass-stoppered bottle.

Boas' reaction depends upon the fact that the addition of aldehyd to Nessler's solution causes a yellowish or reddish precipitate, the intensity of such color bearing a direct relation to the quantity of aldehyd added. This test is extremely delicate, detecting one part of aldehyd in 40,000 parts of water.

Procedure.—1. Ascertain the presence or absence of free acids by employing the Congo-red test.

2. In the presence of free acids add an excess of barium carbonate; evaporate 10 c.c. of the filtered gastric contents to a syrupy consistence over a water-bath.

3. Add a few drops of phosphoric acid to the mixture and boil, when the carbon dixoid present escapes.

4. Remove the mixture from the flame, cool, and add to it 100 c.c. of neutral, alcohol-free ether; after which the mixture is shaken at frequent intervals for an hour.

5. Pour off the ether, evaporate, and take up the residue with 45 c.c. of water.

6. The mixture is now shaken thoroughly, filtered, and 5 c.c. of sulphuric acid and a small amount of manganese dioxid are added to the filtrate.

7. Place in an Erlenmeyer flask—a flask closed by a perforated stopper, through which perforation passes a two-limbed glass tube. The longer limb connects with a cylinder into which 10 c.c. of Nessler's reagent have been placed. The mixture is gradually heated to the boiling-point, when aldehyd, resulting from the oxidation of lactic acid in the presence of manganese dioxid and sulphuric acid, passes over and induces a yellowish-red precipitate—aldehyd of mercury.

Should an alkaline solution of iodopotassic iodid be placed in the cylinder instead of Nessler's reagent, iodoform is precipitated.

Quantitative Estimation of Lactic Acid.—The principles outlined in the preceding qualitative test for lactic acid have been applied by Boas for quantitative analysis. The reader is, therefore, referred to the various steps in the former test while studying this method.

Reagents.—(*a*) One-tenth normal solution of iodin—12.65 gm. (194.4 gr.), one-tenth of the molecular weight of iodin to the liter.

(*b*) One-tenth normal solution of sodium thiosulphate—24.8 gm. (382.7136 gr.), molecular weight, to the liter.

(*c*) A solution of potassium hydrate—56 parts of the salt to 1000 of water.

(*d*) Hydrochloric acid, of a specific gravity of 1.018.

(*e*) Starch solution: dissolve 5 gm. (77.16 gr.) of starch in 100 c.c. of warm water. Dissolve 10 gm. (154.32 gr.) of zinc chlorid in 100 c.c. of water, and add this mixture to the warm starch solution.

Method.—The reaction is carried out as described under *Boas' Reaction* (page 346, steps 1-6).

7. Place in an Erlenmeyer flask, the glass stopper of which

is provided with two openings. Through one opening passes a bent tube which is connected with the distilling apparatus. The other opening admits a glass tube to which is attached a rubber hose, tightly clamped.

8. Distil until from three-fourths to four-fifths of the mixture has passed over, avoiding excessive heat, lest the aldehyd be decomposed.

9. To the distillate add 20 c.c. of a $\frac{1}{10}$ normal iodin solution, with which has been mixed 20 c.c. of a potassium hydrate solution. Shake this mixture thoroughly, and permit it to stand for from five to ten minutes.

10. The excess of iodin is liberated by adding 20 c.c. of hydrochloric acid; and this excess determined by titration with a $\frac{1}{10}$ normal solution of sodium thiosulphate. Continue the titration until the mixture is nearly decolorized, when add a small quantity of the starch mixture and continue the titration until the blue color caused by the addition of the starch has entirely disappeared.

Computation.—Deduct from the number of cubic centimeters of the normal solution employed (20) the number of cubic centimeters of the sodium thiosulphate that were required, and this difference indicates the number of cubic centimeters of the $\frac{1}{10}$ normal solution needed for the formation of iodoform, or the amount of lactic acid that was contained in the 10 c.c. of the gastric contents examined.

One cubic centimeter of the $\frac{1}{10}$ normal solution of iodin represents 0.003388 gm. of lactic acid; therefore multiply the number of cubic centimeters employed by this figure, and the product multiplied by 10 equals the percentage of lactic acid present.

Clinical Significance.—Normally appreciable amounts of lactic acid are not present in the gastric contents recovered during digestion. Lactic acid may be ingested with the food.

A decided reaction for lactic acid points to diminution or an absence of hydrochloric acid (see *Clinical Significance of Hydrochloric Acid*, page 326).

ACETONE.

Pathologic gastric fluid may at times contain acetone. This finding is evidence favoring primary disease of the stomach or intestine, and is not uncommon in gastric cancer.

Acetone is detected in this situation by the application of the Reynolds-Gunning test to the distillate; or the filtrate may be tested according to Denning's method (see *Urine*, page 240).

GASES.

A variable quantity of gas is always found to occupy the stomach, and this gas may have been swallowed or may have entered the stomach from the duodenum. Again, fermentative processes may result in the generation of gases after the liberal ingestion of carbohydrates or of fats. During the processes of proteid digestion a slight quantity of nitrogen, oxygen, and carbon dioxid is to be found in the stomach. Oxygen, when swallowed, is for the most part taken up by the blood, in return for which the blood gives up carbon dioxid in double quantity. This physiologic feature serves to explain why carbon dioxid is often present in large quantities.

The processes of fermentation concerned in the production of both acetic and lactic acids are not capable of forming gas. The processes of fermentation interested in the production of butyric acid give rise to the formation of hydrogen and carbon dioxid. Rarely, under pathologic conditions, marsh-gas results from the fermentation of cellulose, but it is questionable whether this gas, when present in the stomach, has not gained entrance to this viscus from the intestine. The colon bacillus may at times be concerned in gas-production. Sulphureted hydrogen may result from putrefying albuminates, and its presence is suggestive of gastric dilatation, carcinoma, and less often it is found in acute gastritis. Gas is absent when lactic acid is present in moderate amount.

Method.—1. Fill a Doremus ureometer with the fresh, unfiltered gastric fluid, and place at a temperature of 40° to 45° C. (104° to 113° F.) (see *Urine*, page 194).

2. When gas-formation has ceased, introduce into the vertical limb of the tube a small quantity of concentrated sodium-hydrate solution. Should the gas present be carbon dioxid, the liquid ascends.

3. Other gas or gases are estimated from the reading after the sodium hydrate has been added.

4. Sulphureted hydrogen emits its characteristic odor, and turns filter-paper that has been moistened with sodium hydrate and lead acetate a brown or blackish color.

Clinical Significance.—Sulphureted hydrogen gas is a not uncommon feature of the gastric fluid in gastric dilatation, acute gastritis, and gastric cancer.

Where hydrogen, carbon dioxid, and ammonia are present in large quantities, it points to a deficiency of the normal gastric secretion.

MICROSCOPIC STUDY OF THE GASTRIC FLUID.

Gastric fluid recovered from the stomach apart from the process of digestion will be found to consist, for the most part, of mucus and of saliva. Upon microscopic examination, a two-third objective being employed, there are usually detected particles of undigested food; these particles, when brought into focus under a one-sixth objective, will be found to consist largely of elastic fibers. Intimately surrounding these particles of tissue are to be seen muscle-fibers, fat-droplets, crystals of fatty acids, vegetable fibers, starch-granules, leukocytes, mucus-corpuscles, epithelial cells, free nuclei, and granular débris (Fig. 138).

The stomach-contents may be found to contain epithelial cells recovered from the mouth, esophagus, or stomach, and cells derived from the ducts or from the glands and supposed goblet-cells are occasionally found.

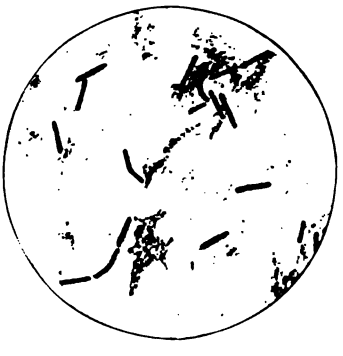


Fig. 137.—Boas-Oppler bacillus from near top of fluid from washing in case of gastric cancer. Observation at Pennsylvania Hospital.

Bacteria.—The bacteria present in the gastric contents are, as a rule, of no clinical significance; yet numerous micro-organisms are at times present, among which may be mentioned the Leptothrix buccalis, various cocci, the saccharomyces, Bacillus subtilis, and the Boas-Oppler bacillus.

The **Boas-Oppler bacillus** is quite constantly present in the gastric contents during the course of gastric carcinoma. This bacillus (Fig. 137) appears in long, segmented chains, which often assume a somewhat tortuous course; stains readily with the ordinary anilin dyes, and is not motile. This bacillus is found present in the gastric contents where free hydrochloric acid is diminished or absent, and where lactic acid abounds. The question has arisen as to whether or not the Boas-Oppler bacillus is concerned in the production of lactic acid. It is not uncommon to find rather dense aggregations and clumps of these bacilli disseminated throughout the gastric contents.

Clinical Significance.—They are fairly constant in gastric fluid whenever lactic acid is plentiful, but they have not been shown to be concerned in the etiology of gastric carcinoma.

Sarcinæ Ventriculi.—These organisms occur in the gastric contents and are arranged in squares or packets; they are to be seen scattered throughout the gastric contents, and when studied singly, they resemble somewhat a miniature bale of cotton (Fig. 138).

Clinical Significance.—The gastric contents may be found to contain few sarcinæ during health. They are also found early during carcinoma, and in pyloric carcinoma where there is yet a secretion of hydrochloric acid. Sarcinæ are seldom observed in advanced gastric carcinoma. Their presence in the gastric fluid is suggestive of a distinct variety of fermentation, yet they are by no means constant where gastric fermentation exists.

Bile.—The vomit may often be stained with bile, and upon microscopic examination such vomit is likely to display crystals of cholesterin, leucin, tyrosin, and biliary pigments. The microchemic reactions for the determination of these substances have been described in the Chapter on *Urine* (page 239).

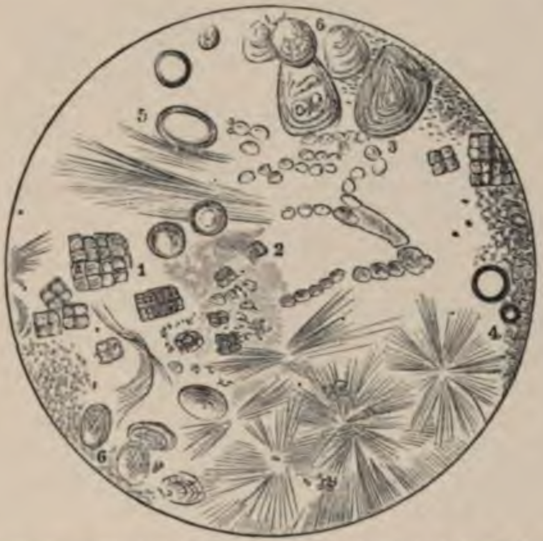


Fig. 138.—Stomach-contents: 1, 2, Sarcinæ ventriculi; 3, yeast; 4, needles of fatty acids; 5, fat-droplets; 6, starch-granules (Riegel).

Tissues.—Many writers have described in detail the finding of shreds of tissue, particles of tumors, etc., recovered through the stomach-tube, and such portions of tumors or sloughing ulcers are of great diagnostic value when thus obtained. In case the piece of tissue be very small, it is well to place a portion of it upon a slide, tease thoroughly with a needle, and then mount in water, applying rather firm pressure to the cover-glass. In this way it is often possible to secure a rather satisfactory specimen, which in the case of sloughing gastric cancer may be composed largely of epithelial cells and will display the features common to cancer. If there be sufficient tissue recovered, it should be hardened and studied in sections (see works on pathology).

It is also possible, though rare, to recover shreds of mucous membrane from cases of chronic gastritis and from neurasthenic individuals. Such tissue, whenever recovered, must, of course, be studied microscopically, and careful note be made as to the presence or absence of nests of cancer-cells, etc. A fact ever to be borne in mind is that gastric carcinoma may exist, and at the same time there be a slight sloughing of the mucous membrane elsewhere. It is not possible, therefore, to exclude the presence of cancer where tissues recovered are not found to be cancerous. Again, tissue that has been subjected to the action of the gastric juice for some time is unfit for microscopic study. In my own experience it has been extremely rare to recover particles of tissue from the stomach-wall, and I do not recall a single instance where a diagnosis was attained through the study of such particles of tissue.

THE VOMIT.

Odor.—Normal gastric juice has a characteristic odor, which, while it resembles that of acids, is unlike any other well-known odor. Pathologic gastric fluid may emit an odor, due to the presence of certain gases previously described (page 349). A putrid odor is suggestive of pyloric stenosis, with or without ulceration of the gastric surface. Following the ingestion of certain foods, the gastric contents will be found to display the odor known to such foods. The odor of ammonia is emitted by the gastric contents of uremic persons, and after the administration of toxic doses of phosphorus the gastric fluid will give off an odor of garlic. Carbolic acid also lends its odor to the vomit.

The vomit will vary greatly, depending upon the conditions by which it is excited; the preëxistence of nausea; the material taken in the stomach prior to vomiting, and whether or not the vomit contains bile, saliva, mucus, blood, or fecal material. Again, material may seemingly be vomited which, in reality, is simply regurgitated from the esophagus.

Vomiting may occur immediately after the ingestion of food, some hours after taking food, or upon rising; which influences materially alter the character of the vomit. Large quantities of undigested food may be vomited two or more hours after the ingestion of a meal, but this observation is rare and occurs in cirrhosis of the stomach. There is at times a regurgitation of food, mucus, and saliva where stricture of the esophagus or of the cardiac portion of the stomach exists, but such regurgitation, as a rule, occurs soon after the taking of food. It is not uncommon, however, to find food regurgitated some days after it has been taken—a condition seen most often in gastric dilatation. Food regurgitated from the esophagus is distinguished from that ejected from the stomach by the fact that the latter may contain gastric juice and biliary coloring-matter. During the course of gastric neuroses, ulcer, acute gastritis, and early in chronic gastritis, as well as in reflex (spinal) vomit, the food is rather thoroughly digested.

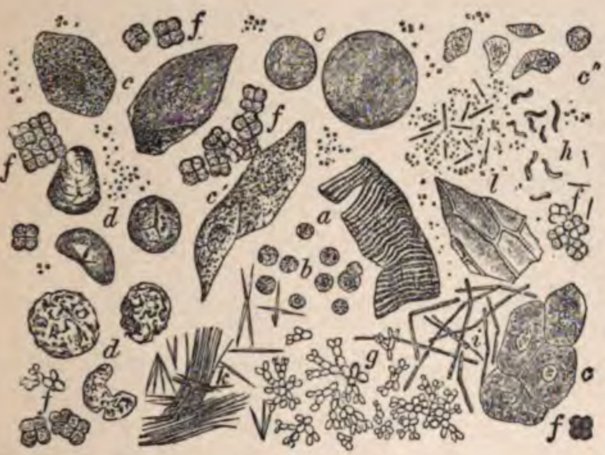


Fig. 139.—Vomited material: *a*, Muscular fiber; *b*, white blood-corpuscles; *c*, *c'*, *c''*, flat and cylindric epithelia; *d*, starch-corpuscles; *e*, fat-globules; *f*, Sarcinæ ventriculi; *g*, yeast ferment; *h*, *i*, cocci and bacilli (those near *h* were once found by von Jaksch in a case of ileus, hence arising from intestine); *k*, fat-needles, connective tissue; *l*, vegetable cells (von Jaksch).

Where the nausea precedes the vomiting, the vomitus will be found to contain a large quantity of mucus and of saliva. It is, therefore, unsafe to form any deductions concerning the amount of mucus found in vomited material.

Saliva.—Persons suffering from chronic catarrhal conditions of the throat may at times vomit almost pure saliva. Reactions for saliva are to be obtained in almost any vomit, and may be detected through the methods outlined under *Saliva* (see page 452).

Mucus.—The presence of mucus in the vomit is of little consequence, but where the gastric contents is obtained through the stomach-tube, large quantities of mucus are to be seen only in pathologic conditions, and are commonly suggestive of the existence of mucous gastritis. It has been claimed that where the gastric contents is purely mucus, gastric dilatation exists. Large amounts of mucus may at times collect in the stomach between the stages of digestion, but this condition is certainly rare. In addition to the characteristic appearance which mucus lends to the gastric contents, it will be found to react characteristically with acetic acid (see *Urine*, page 262).

Bile.—The vomit is commonly colored, due to the presence of bile, but it is unusual to find bile in the gastric contents that has been recovered through the stomach-tube, and I recall but a single instance, that of a neurasthenic female of thirty-five years.

Biliary vomit, when frequent, points to the existence of obstruction to the lower duodenum or upper portion of the jejunum. The presence of bile in the gastric fluid is in a measure indicative of pancreatic disease.

Stercoraceous Vomit.—Stercoraceous material, when vomited, is at once recognized by its odor (fecal). It is found to contain phenol, indol, and skatol (see *Feces*, page 366). Bile and bile acids may also be present in the fecal vomit, which is, as a rule, alkaline in reaction and occurs in intestinal obstruction.

Blood.—The vomit may contain blood in a number of conditions, many of which induce secondary gastric hemorrhage. Among the primary causes of gastric hemorrhage and the vomiting of the blood are: gastric cancer, ulcer, traumatism, the retching of vomiting, and acute gastritis. It is also found secondary to chronic Bright's disease, valvular heart disease where compensation has been lost, chronic lung disease, cirrhosis of the liver, anemia (pernicious anemia, leukemia, and chlorosis), and, rarely, it is the result of vicarious menstruation.

Detection.—Should the blood be present in large quantities, it will be found to lend its color to the vomit, such color varying in intensity from that of a dirty-brown to a blackish hue. This color may be largely due to the action of the gastric juice upon the blood. It is often possible to detect blood-corpuscles upon microscopic examination of the gastric fluid.

Minute quantities of blood, when present in the gastric juice, are best detected through the method suggested by Müller and Weber: Treat 10 c.c of gastric contents with about 2 c.c. of ether, shaking to effect a mixture. The ether will be found to separate into a clear stratum when the mixture is allowed to stand for a few minutes; should this separation fail to occur, add a few drops of alcohol. The addition of ether causes, in the presence of blood-pigment, a brownish-red color to develop, which is dependent upon acetate of hematin. Bile-pigment causes a yellowish-brown color. The guaiacum test (page 31) should also be applied to the ethereal extract. Spectroscopic study, however, is scarcely practical.

Klunge's Aloin Test.—Dissolve as much aloin as will rest on the tip of an ordinary spatula in 10 c.c. of 70 per cent. alcohol.

1. Treat the gastric fluid or feces as directed by Müller and Weber, except pour away the ether when it collects at the top after the first treatment.
2. Place 2 c.c. of the ethereal extract in a test-tube and to it add 2 c.c. of the aloin reagent. Add drop by drop, shaking after each addition, ten to twenty drops of Merck's oil of turpentine.
3. Stand aside for a few minutes, when should blood be present the lower half of the liquid becomes a rich cherry-red color. In the absence of blood a violet color develops.

Clinical Significance.—In cancer of the stomach a positive reaction is obtained with both gastric fluid and with feces. Benign gastric and duodenal tumors and gastric ulcer show an intermittent positive reaction with the feces. Negative results are obtained with the feces in cases of gastritis acidi, hyperacidity, neuroses and benign dilatation.

Pus.—It has not been my privilege to detect pus in the gastric contents obtained through the stomach-tube. It is to be detected, however, in cases of diphtheric gastritis, in gastric carcinoma located at the fundus and lesser curvature, and, according to certain authors, the recovery of a dram or more of pus through the stomach-tube is of diagnostic value in this disease; but should a large quantity of pus be obtained, it would doubtless have gained access to the stomach through a fistulous opening, and might come from the diaphragm, liver, pleura, lung, or any of the adjacent viscera.

Pus is distinguished by its microscopic appearance.

Parasites.—I have found the ascaris, oxyuris, and segments of the tape-worm in the vomit. Other observers have detected the ankylostomum, trichinæ, and, according to Simon, the Trichomonas vaginalis has been found present in the vomit from a case of esophageal cancer.

CHAPTER IV.

THE FECES.

By an examination of the feces we are able to determine the character of this discharge, and to draw certain conclusions as to the nature of intestinal digestion or of any derangements that may be present; provided that these derangements are accompanied by abnormalities in either the physical or the chemic properties of the feces. At times the feces may contain definite products of disease, or the pathogenic organisms of disease may be present: for example, the intestinal animal parasites and the organisms producing dysentery.

It is impossible to obtain much clinical evidence from the study of the feces, because we are dealing with the last step of an extremely tedious and complicated process, the exact nature of the previous steps of which is not yet thoroughly understood. It is, therefore, possible for a slight pathologic variation to exist in the feces, and for this variation to escape our notice. Again, the feces may vary within wide limits during health, as to both their physical and their chemic properties, and this fact renders it extremely difficult to draw well-balanced deductions, except in connection with a few maladies. The bulk of the feces will be found to consist of undigested particles of food and of unabsorbed secretions furnished by the various glands that empty into the gastro-intestinal canal, to which may be added a variable amount of mucus, epithelial cells, and bacteria.

COLLECTION OF THE FECES.

It is preferable to have the feces passed into a well-warmed pan or vessel, which should be sent to the laboratory as soon as possible, so that the specimen does not become chilled. It is of especial importance that the specimen be kept warm when we are searching for the Amœba coli or any other motile animal parasite. Should the specimen be collected from a patient at his residence, it should be passed into a warm vessel, as previously stated, and whenever a portion of the feces is desired for clinical study, this should be placed in a wide-mouthed bottle, which should be corked tightly and kept as warm as possible while being carried to the laboratory. As soon as the specimen is received in the laboratory, the tightly corked bottle should be placed in an incubator at a temperature of 37° C. (98.6° F.), where it should be kept until the time for its examination.

GENERAL CHARACTERS OF THE FECES DURING HEALTH AND DURING DISEASE.

Frequency.—The frequency of the intestinal discharges will be found to vary considerably in the adult. Children upon a milk diet usually have three or four evacuations of the bowel daily. Adults and children upon solid food have, as a rule, one or two stools in twenty-four hours. There are certain individuals who may have three, or even four, movements daily, and, on the other hand, it is not unusual to find persons who have a movement of the bowels only once or twice a week. In one case under personal observation the patient claimed that he had a rather large stool once in every eight days. This man was sixty-four years of age, a surveyor by occupation, and, from his occupation, was constantly walking over the mountains of the eastern United States. He died of pneumonia at the age of sixty-four years, his final illness being the first time he had called on a physician for professional services.

Pathologic Frequency.—Pathologic constipation may result from a variety of diseases; but, in a general way, maladies beginning abruptly are accompanied by constipation, except cholera and cholera morbus. In typhoid fever and in dysentery, in which frequent stools are characteristic, the diarrhea is preceded by constipation. It is impossible to say that any person is suffering either from diarrhea or from constipation unless the physician is acquainted with the normal number of bowel movements for the given individual. The quantity of material discharged at each movement is likely materially to influence the number of the discharges. For example, in acute dysentery the number of movements may vary from 20 to 200, or even more, daily, but the quantity discharged at each operation is extremely small and may not exceed one dram. The character and the quantity of the food taken will also be found to influence materially the frequency and the quantity of the intestinal discharges. The grade of the peristalsis also influences the number of stools—rapid peristalsis exciting frequent bowel movements, while sluggish peristaltic waves favor obstipation. Interference with the lumen of the intestinal canal, from whatever cause, induces constipation or obstruction, depending upon the degree to which the caliber of the intestine is reduced.

Clinical Significance.—Diarrhea, when present, is significant of intestinal catarrh. Such an inflammatory process of the intestine may be the result of errors in diet, exposure to cold, or the growth of some infectious agent: *e. g.*, Bacillus typhosus, Amœba coli, Bacillus dysenteriæ. Whatever the cause, it is to be remembered that the lesions involve the mucosa of the large intestine.

The stools are always thin, more or less watery, and in certain diseases they have some characteristic features: *e. g.*, the pea-soup stool of typhoid fever, and the mucoserous and mucoseropurulent stools of acute dysentery. These stools may occur every few hours or more frequently. The stools of Asiatic cholera are characterized by their rice-water appearance. The liquid portion of such stools is derived from the patient's blood-serum.

Drugs.—Drugs may also excite frequent bowel movements, and may at times cause a true intestinal catarrh. In such a condition the stools closely resemble those of acute dysentery.

Constipation.—Obstinate constipation, while it is extremely troublesome and annoying, may not be of serious moment. It is an early and valuable diagnostic sign of peritonitis and paralysis of the intestine. It is further suggestive of stenosis of the intestine, fecal impaction, or complete obstruction of the bowel. Constipation with the passage of ribbon-like stools points definitely to a progressive lessening of the lumen of the intestine.

In amebic dysentery there may be constipation which alternates with exacerbations of diarrhea—six to twelve movements daily. It is impossible to draw any universal conclusions concerning the various degrees of constipation, since each case is practically a law unto itself. Constipation may form a prominent feature of esophageal carcinoma and of malignant disease of the stomach or the intestine. In such cases it is usually the result of starvation.

Amount.—Under normal conditions the amount of feces dejected during twenty-four hours will be found to vary between 60 and 250 gm., an average of from 100 to 200 gm. The wide degree of fluctuation is directly influenced by the quantity and the character of the food ingested, the degree of intestinal irritation, and the frequency of the peristaltic waves. Under pathologic conditions it is not unusual to find from 500 to 1200 gm. of excreta discharged daily. Protracted constipation or a temporary intestinal obstruction may be followed by a copious discharge of feces.

Form.—In health the stool is rather firm or mushy, its consistence depending directly upon the quantity of water which it contains, and this quantity of liquid is likewise governed by the character of the food and the quantity of the liquids ingested. When the patient is placed upon a vegetable diet, the stools will be found to contain about 80 per cent. of water; when upon a mixed diet, the quantity of water present is somewhat lessened; and when the diet is formed principally of animal proteids, the dejecta may contain not more than 60 per cent. of water.

Scybalous masses are seldom observed under normal conditions, but are commonly seen in the bowel movements some hours before the development of diarrhea. Thin, tape-like or ribbon-like stools are often indicative of rectal stenosis (see *Constipation*, page 358).

If a stool is permitted to stand in a vessel for some time, the watery element occupies the upper stratum, while the solid constituents are thrown down as a precipitate. Such stools are characteristic of intestinal catarrh, and are best seen during typhoid fever, although this condition may be observed whenever the feces are composed largely of water. It is to be remembered that such a peculiarity may be favored by the admixture of urine.

Odor.—The odor of the stools may be somewhat characteristic of the disease affecting the alimentary tract, or it may be altered by the food or by medicines (onions, asafetida) that have been taken. As a rule, the odor is dependent upon indol and skatol present in the feces, although at times a decided odor of sulphureted hydrogen may be detected, and, rarely, the odor of methane or of phosphin.

Pathologic Odor.—The following alterations in the odor of the stools have been noted during pathologic conditions. In persons suffering from acute or protracted alcoholism the odor of the stools is extremely foul. The dejecta of children, when acid fermentation has been going on in the intestinal tract, often emit the odor of fatty acids. When alkaline fermentation has taken place in the presence of albuminous material, the stools may have a putrid odor. The admixture of blood and pus with the feces may produce a rather heavy odor, resembling that of rancid butter. The feces will be found to give off an ammoniacal odor when a fistulous communication with the bladder permits the escape of urine into the bowel. Cadaver-like smelling stools are characteristic of gangrenous processes along the alimentary tract, usually affecting the large bowel. In cases of Asiatic cholera the stools emit a spermy odor which is probably due to the presence of cadaverin. In cases of tuberculous ulceration of the bowel a decidedly foul odor is present. In acu catarrhal dysentery, when there are frequent stools composed of small quantity of mucus, blood, and pus, there may be no od to the feces, but there is usually an escape of a large amount fetid gas with each fecal discharge. In a case of amebic dysentery under personal observation the feces had a sweetish odor.

Color of Stools.—Under normal conditions the color of th feces will be found to vary, in accordance with the character o the food taken, from a light yellow to a brownish black or blackish blue. Usually the more solid the consistence of the feces, the darker the color, except when the excessive color is directly dependent upon the food taken. Exposure to the air and light causes the stools to become darker than they were when passed. In health, huckleberries produce blackish stools; chocolate gives the feces a dull-gray color; cocoa produces a light-gray shade; chlorophyl colors them green; and starches produce a yellow color; fats, too, cause a clay color.

Hemorrhage.—After hemorrhage from the bowel the feces may be blood-red in color; but when the blood has gradually oozed into the bowel for a long period, the stools become black, on account of the formation of hematin. In other cases hemorrhage from the stomach, from typhoid ulceration, or from other forms of intestinal lesion is followed by deep black stools, from the formation of sulphid of iron. When the hemorrhage is rather profuse,—say a few ounces of blood,—the stools are tar-like in color and in consistence. As a rule, the higher in the alimentary tract the seat of the hemorrhage, the darker the color of the feces; thus, coffee-colored stools are seen after gastric and duodenal hemorrhages. Fresh blood, when adherent to scybalous masses or to solid feces, is usually from the rectum and anus.

Tests for Blood.—In case it is impossible to detect blood-cells in the feces by the microscope, one must resort to the special chemic tests for the presence of blood-pigments. Such clinical observation is, however, more scientific than practical in value. Minimum quantities of blood, when present in the feces, may be detected by the method suggested by Müller and Weber (see *Gastric Contents*, page 354, and *Blood*, p. 31).

Karczynski-Jaworski Test.—1. Place a small amount of the feces in a porcelain dish, add a smaller amount of potassium chlorate, and one drop of hydrochloric acid (c. p.).

2. Heat carefully over the flame of a Bunsen burner until the mixture is decolorized (the addition of one or more drops of hydrochloric acid may be required). During this process chlorin escapes.

3. Add from one to five drops of a dilute solution of potassium ferrocyanid; the appearance of a distinct Prussian-blue color indicates the presence of blood-pigment.

Fallacies.—Pus, when present in the feces in appreciable amount, will produce the same reaction with potassium ferrocyanid. See also *Klunge's Aloin Test*, p. 355.

Drugs.—The various preparations of iron, bismuth, and manganese dioxid, when administered by the mouth, produce a dark-brown or black color of the feces. This color is dependent upon the formation of sulphids of these metals. The stools assume a decidedly greenish hue after the administration of calomel, which is probably due to an excess of biliverdin in the intestine. Ipecac produces clay-colored stools; while senna and santonin, when administered in physiologic doses, give a yellow color to the feces.

Bilious Stools.—The so-called "bilious stools" vary in color from bright yellow to dark green. This color is due to the escape of unchanged or but slightly altered bile which has passed hurriedly through the bowel. Such feces may be filtered, and the filtrate tested for the presence of bile (see *Urine*, page 239). When the bile-supply is cut off from the intestine, the feces soon become of a clay color and have a peculiar glistening appearance. This glistening appearance of the feces is doubtless due, in a measure, to the lack of bile; but to a certain extent it is caused by the small particles of fat that are more or less thoroughly disseminated throughout the mass, not having been absorbed by the intestine.

Clay-colored Stools.—Clay-colored stools are found in conjunction with all forms of obstructive jaundice (see *Fatty Stools*); but the feces from a patient with obstructive jaundice have a light-brown color if the patient is kept upon a diet containing a minimum amount of fat.

It has been proved that the lack of color of the feces is not always due to the absence of bile nor to the presence of fats; and it is, therefore, supposed that the decomposition of urobilin may result in the formation of decolorizing products. Nencki has described a substance which he called "leukourobilin"; and it is possible that some allied product or products are concerned in decolorization of the feces.

Green stools may be due to the development of the Bacillus pyocyaneus or other bacteria which form a green chromogen. There is some reason for believing that the feces may at times owe their color, in part at least, to the development of other chromogenic bacteria.

Red Stools.—Carter and MacMun have reported three instances in which the feces turned red upon exposure to the air and light. It is suggested by the latter of these writers that the chromogen here concerned is closely allied to stercobilin. In this connection it may be profitable to cite a series of experiments conducted at the Philadelphia Hospital in collaboration with Dr. Charles A. Oliver, and reported by the latter.* These experiments showed that the chromogens resulting from the development of the different chromogenic bacteria produced a different shade, and often an entirely different color, when grown under colored glass plates: *e. g.*, red, green, blue, and yellow. When kept in the dark, little or no color develops. I am inclined to believe that feces that become red on exposure to the light owe this redness to the chromogens of bacterial development—Bacillus prodigiosus, Micrococcus prodigiosus, etc.

CHEMISTRY OF THE FECES.

In American laboratories there appears to be but a small amount of time devoted to the study of the chemistry of the feces. Sufficient has been done, however, to permit of definite deductions regarding the chemistry of normal feces; but the data are by far too small to justify conclusions regarding the chemistry of the feces in disease.

REACTION OF THE STOOLS.

The reaction of the stools of a normal adult is, as a rule, alkaline; but it is sometimes neutral and occasionally acid. The alkalinity of the normal feces is dependent upon ammoniacal fermentation. The acidity is due to the presence of lactic or of butyric acid, the formation of either of which may occur, by fermentation, in the intestine. The feces of children when taking a milk diet should, under normal conditions, give a slightly acid reaction.

Clinical Significance.—In the adult, whenever the feces are decidedly acid, the reaction is indicative of intestinal catarrh with the production of lactic- and butyric-acid fermentation in the intestine. When the feces of children are found to be highly acid, the existence of an intestinal catarrh may be inferred; and in the adult the acidity depends upon acid fermentation. A decided alkaline reaction may depend upon the occurrence of alkaline fermentation; it is usually indicated by its characteristic odor (see *Odor*, page 359). The reaction of the feces is of limited diagnostic value.

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*"Amer. Jour. Med. Sci.," April, 1902.

COMPOSITION OF THE FECES.

Dunglison credits Simon with the following as the composition of normal feces:

Water	77.3	per cent.
Mucin	2.3	" "
Proteids	5.4	" "
Extractives	1.8	" "
Fats	1.5	" "
Salts	1.8	" "
Resinous, biliary, and coloring-matters	5.2	" "
Insoluble residue of food	4.7	" "

Gautier gives the following table regarding the composition of the freshly passed feces (Gautier's estimations are based upon 1000 parts by weight):

	Adult Man.	Suckling.
Water	733.00	851.3
Solids	267.00	148.7
Total organic material	208.75	137.1—including 54 parts of mucin, epithelium, and calcareous salts.
Total mineral material	10.95*	13.6
Alimentary residue	83.00	

The organic material yielded:

	Man.	Suckling
Aqueous extract	53.40	53.5
Alcoholic extract	41.65	8.2
Ethereal extract	30.70	17.6—of which 2.2 is cholesterin.

In a general way it is possible to gain a fairly clear, concise, and at the same time general idea of the feces by considering under the following headings the various chemic constituents present:

1. *Unassimilated Food.*—Probably the greater portion of the solid and semisolid materials of the feces consists of food that has not been assimilated, but which, if taken in proper amounts into a stomach during health, should have been assimilated. This class of food-material appears in the feces oftenest as a result of overeating. These food-materials consist for the most part of starches, albuminous materials, and fats.

2. *Indigestible Matter.*—A varying proportion of the feces will be composed of substances not capable of digestion, and hence incapable of assimilation; among which class are to be found gums, resins, gelatinous substances, chlorophyl, pigments, nucleins, and the various insoluble salts.

3. *Substances from the Digestive Tract.*—A portion of the feces will be seen to consist of organic substance derived from the gastro-intestinal tract and from the adjacent glands which empty

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* Not comprising earthy phosphates.

their secretions into the intestine, such as epithelial cells, mucus, bile acids in various stages of transformation, cholesterin, and lecithin.

4. The feces also contain substances practically ready for absorption, but which, owing to peristaltic movements, have been hastened through the bowel before such absorption could take place; these are emulsified fats, fatty acids, biliary acids, and leucin.

5. *Products of Metabolism.*—A variable amount of the feces is dependent upon metabolism, such metabolic products being eliminated through the alimentary tract. In this connection urea, uric acid, and the various xanthin bases should be considered, although these substances are not eliminated by the feces in amounts sufficient to warrant their special study in this excretion.

6. *Purin Bodies.*—For description see page 533.

7. *Coloring-matter.*—A small amount of coloring-matter is also found in the feces. It consists of the modified pigments of the blood and of the bile—hematin, stercobilin, and hydrobilirubin. Hematoidin, pure blood, and the unaltered bile-pigments are abnormal constituents of the feces.

8. *Decomposition Products.*—The products of decomposition dependent upon bacterial development, such as the various fatty acids, butyric and isobutyric acid, lactic acid, amido-acids, acid amids, leucin, tyrosin, phenyl-acetic acid, phenol, skatol, excretin, indol, and cresol, are constituents of the feces. Both ammonium carbonate and ammonium sulphid are produced by processes of decomposition.

9. *Gases.*—The gases present in the feces during health vary in direct proportion to the character of the food ingested. The formation of gas may be a feature of certain diseases. Under normal conditions carbon dioxid, hydrogen, nitrogen, and marsh-gas appear in the feces. The carbon dioxid present is dependent upon alcoholic fermentation, butyric-acid fermentation, and upon the putrefaction of albumins which take place within the intestine. Theoretically, carbon dioxid is supposed to pass directly from the blood into the intestine.

Nitrogen may be swallowed, but a portion of this gas found in the feces is probably evolved from putrefying albumins. Glucose, while undergoing fermentation, generates a certain percentage of marsh-gas.

It is reasonable to suppose that a special study of the gases of the feces may enable one to form an approximate idea of the putrefactive and fermentative changes taking place in the intestinal tract; but as yet a practical clinical method for the quantitative and qualitative detection of such gases has not been proposed.

I shall consider briefly the chemistry of the biliary acids, the pigments, the fatty acids, and cholesterin, and shall mention certain other substances known to occur in normal feces. Bloody stools have been discussed under a separate heading.

TYROSIN.

Tyrosin is conceded to be the mother-substance of phenol, indol, skatol, and cresol. Tyrosin, when present in the gastro-intestinal tract, is the result of the putrefaction of albumins. It is readily destroyed in the intestinal tract after it is produced, with the formation of phenol, indol, skatol, and cresol, which may be found in the feces. Tyrosin itself is rarely found in this excretion (see *Urine*, page 268). Phenol, indol, skatol, and cresol may be detected in the feces by the following reactions:

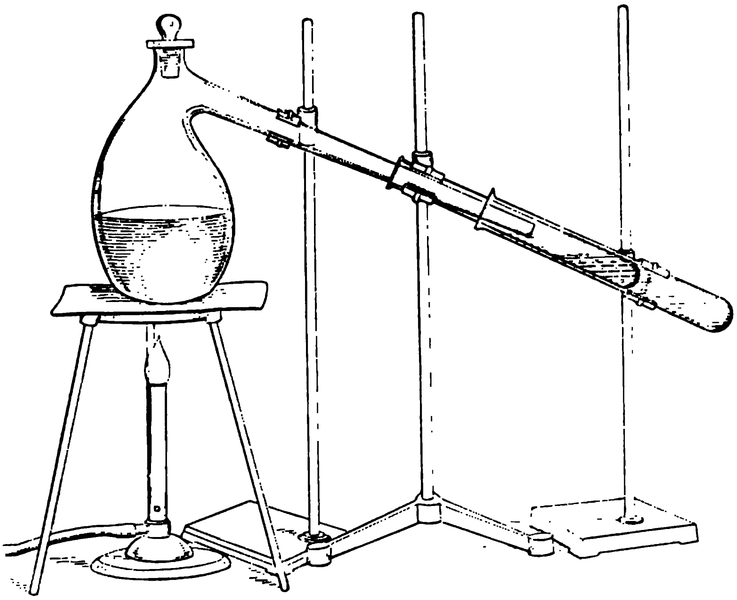

Fig. 140.—Distilling apparatus.

Method of Detection.—1. The feces should first be well diluted with water, acidified with phosphoric acid, and then distilled. By this process all the volatile fatty acids, indol, skatol, phenol, and cresol will be forced into the distillate.

2. Neutralize the distillate with sodium carbonate and distil

again. During this second distillation the fatty acids are left behind as sodium salts, while indol, skatol, phenol, and cresol pass into the receiver.

3. Phenol may be separated from indol, skatol, and cresol by rendering the second distillate alkaline with potassium hydroxid and redistilling. During this third distillation indol, skatol, and cresol pass over, while phenol is left behind. According to Marshall, this precipitate is tribromphenolbrom or tribromobromphenol.

4. The residue in the retort should be distilled with sulphuric acid, and pure phenol will be forced over in the distillate, as may be shown by the following reactions:

Phenol.—(*a*) Place a portion of the distillate in a test-tube and add a few drops of a solution of iron perchlorid. In the presence of phenol an amethyst-blue color develops (see *Tests for Lactic Acid*, page 345).

(*b*) Place a few cubic centimeters of the distillate in a test-tube and add an equal volume of bromin water. In the presence of phenol a crystalline precipitate of tribromphenol is formed.

(*c*) The distillate, when treated with Millon's reagent (see *Urine*, page 217), shows a decided red color when phenol is present, due to the formation of acid nitrate of mercury.

Indol.—In order to separate indol from skatol it is necessary to observe their degrees of solubility closely. Small plates of indol are liquefied at 52° C. (125.6° F.). They are also readily soluble in hot water, alcohol, and ether. During this process the mixture will be found to emit a feculent odor.

(*a*) Indol, when treated with nitric acid and a variable amount of sodium nitrate, gives a red, crystalline precipitate of nitrosoindol nitrate.

(*b*) Take a small piece of a clean pine stick and moisten it with an alcoholic solution of indol; then plunge the stick into a solution of hydrochloric acid. The wood will change to a cherry-red color if indol be present.

Skatol.—(*a*) Crystalline skatol appears in quite regular plates which dissolve at a temperature of 95° C. (203° F.). Crystals of skatol are less readily soluble than are crystals of indol; but during their solution a similar feculent odor is given off.

(*b*) Skatol, when treated with nitric acid and sodium nitrate, results in an opalescent (milky) mixture.

(*c*) Skatol, when boiled with nitric acid (sp. gr. 1.2), gives the xanthoproteic reaction.

(*d*) The color reaction of hydrochloric acid and pine wood previously dipped in a solution of skatol is different from that produced by hydrochloric acid and pine wood dipped in a solution of indol. First moisten the wood with a weak alcoholic solution of skatol, and then dip it into a strong solution of hydrochloric acid; a cherry-red color, which soon changes to blue or violet, will be produced.

FATTY ACIDS.

All members of the group of fatty acids, from formic acid to stearic acid, may at times be found in the feces.

Derivation.—While a portion of these fatty acids is derived from the fats ingested, it is to be remembered that some of them are derived from the ingested carbohydrates and proteids.

Physical Properties of Fatty Acids.—The fatty acids are monobasic, and are soluble in water, alcohol, and ether. The alkaline salts of the fatty acids are also soluble in water and in alcohol, but are not soluble in ether. The silver salts of the fatty acids are not readily soluble.

Detection.—1. The reader is referred to the special method described under *Tyrosin* for the detection of phenol, indol, skatol, and cresol. If the first distillate obtained from distillation of the feces be neutralized with sodium carbonate and distilled a second time, the fatty acids remain in the retort as their sodium salts.

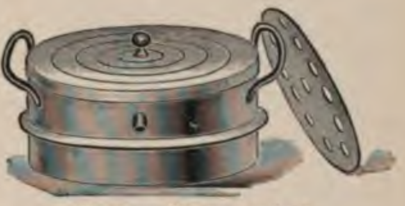

Fig. 141.—Warm-water bath.

2. Evaporate this solution over a water-bath (Fig. 141) to dryness, and extract the residue with alcohol; evaporate the alcohol, and dissolve the residue in water. By this method a solution is obtained that may be employed for the further study of the fatty acids.

Whenever it is desired to separate the different fatty acids, the majority of authors recommend converting them into silver or barium salts, which may be distinguished by their solubility in water. It is possible, however, to employ fractional distillation for the separation of fatty acids.

Formic Acid.—Formic acid is a colorless, pungent liquid which boils at 100° C. (212° F.). To a concentrated solution of the alkaline salts of formic acid add silver nitrate. Upon the application of heat a black precipitate results; but if the mixture be allowed to stand at room-temperature for a time, the silver salt blackens without the application of heat.

Place a neutral solution of formic acid in a test-tube and add a few drops of a solution of iron perchlorid. A blood-red color indicates the presence of formic acid. When this red mixture is heated, its color fades, and finally disappears on boiling. At the same time a rusty-colored precipitate collects at the bottom of the tube.

Acetic Acid.—Acetic acid is a colorless liquid, of a penetrating, vinegar-like odor, boiling at a temperature of 119° C. (246.2° F.).

1. Acetic acid when cautiously neutralized gives a blood-red color after the addition of a few drops of iron perchlorid.

2. A neutral solution of the alkaline salts of acetic acid gives a precipitate when treated with silver nitrate. This precipitate is soluble in hot water, but reduction does not take place.

Butyric Acid.—Butyric acid is an oily substance having an odor like that of rancid butter, and boiling at a temperature of 137° C. (278.6° F.).

1. Solutions of butyric acid and of its salts do *not* turn red when treated with iron perchlorid.

2. Solutions containing the alkaline salts of butyric acid, when treated with silver nitrate, give a precipitate which is insoluble in cold water.

3. Solutions containing the salts of butyric acid emit its characteristic odor upon the addition of acids.

Valerianic Acid.—Valerianic acid is an oily liquid having a pungent odor and boiling at a temperature of 176.3° C. (349.3° F.). Its odor, while disagreeable, can scarcely be said to be characteristic. When treated with solutions of silver nitrate, its silver salts crystallize in rather regular plates which are not readily soluble.

Propionic Acid.—Propionic acid is a sour, pungent, oily substance, boiling at a temperature of 117° C. (242.6° F.). When treated with a dilute solution of iron perchlorid, it does not produce a red color. When treated with silver nitrate, it is practically indistinguishable from formic acid.

BILIARY ACIDS.

Among the biliary acids found in the feces are glycocholic acid, taurocholic acid, and cholalic acid. Glycocholic acid ($C_{26}H_{43}NO_6$) and taurocholic acid ($C_{26}H_{45}NSO_7$) are normal constituents of the bile, and are converted into cholalic acid ($C_{24}H_{40}O_5$) and glycocoll, or cholalic-acid taurin, by processes of decomposition. Cholalic acid is the biliary acid found in the feces, because it is the product of the decomposition of glycocholic and taurocholic acids in the intestinal canal.

Detection.—The detection of biliary acids in the feces can be accomplished only after phenol, indol, skatol, cresol, and the fatty acids have been removed by distillation with phosphoric acid (see *Test* for these substances).

1. After removing the previously named substances from the feces, dissolve the residue with water; boil and filter.

2. Treat the filtrate with lead acetate and a small quantity of ammonium hydroxid until a precipitate forms. This precipitate contains the biliary salts of lead.

3. Wash the precipitate with water, and boil with alcohol to remove the salts of the biliary acids; filter.

4. Treat the final filtrate with sodium carbonate, in order that the lead salts may be transformed into sodium salts; filter again, evaporate this filtrate to dryness, and extract the residue with hot alcohol. The salts of the biliary acids may crystallize, but the rule is to obtain an amorphous, dirty, brownish precipitate.

5. Treat this amorphous precipitate with ether, so as to produce crystalline substances. Crystallization of the amorphous precipitate is not necessary, however, since it is possible to employ this material for further testing.

Pettenkofer's Test.—(*a*) Place a small amount of the amorphous precipitate in a dish, and add sufficient water to dissolve it completely. To this solution add two-thirds its volume of concentrated sulphuric acid; the temperature of the mixture should not be above 70° C. (158° F.).

(*b*) While the mixture is kept warm, add a 10 per cent. solution of saccharose drop by drop. In the presence of biliary acids the entire mixture assumes a beautiful red color which changes to blue or violet on standing.

PIGMENTS OF THE FECES.

Stercobilin and hydrobilirubin deserve special mention in connection with the pigments of normal feces. According to Gautier, stercobilin, which is the principal coloring-matter found in the feces, is derived from bilirubin. According to the reports of a number of chemists, stercobilin and hydrobilirubin appear to be similar, if not identical.

Detection.—Extract the feces with acid alcohol, and to this extract add water and a variable quantity of chloroform. Upon vigorous shaking, the pigment dissolves.

Stercobilin, upon treatment with zinc chlorid and ammonium hydroxid, gives four absorption-bands. Hydrobilirubin, in striking distinction, gives but three absorption-bands when subjected to this treatment (see *Spectroscopy*, page 43). It will be found of interest to compare the spectra of stercobilin, normal urobilin, and hydrobilirubin. The last is probably identical with the so-called febrile urobilin.

CHOLESTERIN.

A trace of cholesterin ($C_{26}H_{44}O$) is to be found in practically all the body-fluids. The method of the production of cholesterin wherever it may appear is undetermined.

Crystals.—Crystals of cholesterin (Fig. 92) appear as thin, colorless, semitransparent plates, one or more margins of which are usually irregular, showing a more or less jagged, imperfect, saw-like edge. The remainder of the margins are usually clearly cut and of regular outline. Crystals of cholesterin are, as a rule, irregularly oblong or square. Crystals from which one corner appears to have been broken are also common. Crystals of cholesterin may occur singly, but it is more common to find them in dense aggregations.

Solubility.—Crystals of cholesterin are soluble in water, dilute acids, and alkalis. They are freely soluble in boiling alcohol, ether, chloroform, and benzol. After solution in hot alcohol cholesterin is precipitated on cooling.

In the feces the cholesterin does not appear in crystalline form except under rare conditions.

Detection.—1. In order to detect cholesterin in the feces the fatty acids, phenol, indol, skatol, and cresol must first be removed by distillation (see *Special Study* of these substances).

2. Next add sulphuric acid to the residue; extract with alcohol, and continue the extraction with ether.

3. Filter the ethereal extract, remove the ether by distillation, and then treat the residue with sodium carbonate, thereby converting any fatty acids present into their sodium salts.

4. Evaporate the mixture to dryness and extract with ether.

5. Filter the previously prepared alcoholic extract, and then supersaturate the filtrate with sodium carbonate; remove the alcohol by distillation; dissolve the residue in water, and continue the extraction with ether. By this process any bile acids, oleic acid, stearic acid, etc., separated by further analysis, may be converted into their respective barium salts (see *Fatty Acids*, page 367).

6. Cholesterin and the fats are dissolved in the ether. Place this final solution in a beaker and evaporate over a water-bath; treat the residue with an alcoholic solution of potassium hydroxid.

7. Evaporate this alcoholic solution over a water-bath (Fig. 141), dilute the remaining fluid with water, and extract with ether.

All fats present will be found suspended in the aqueous solution as soaps. The cholesterin, on the other hand, will have been dissolved in the other.

(*a*) Place the suspected solution on a slide, add a drop of water, apply a cover-glass, and examine with a one-fifth or one-sixth objective. Crystals of cholesterin (Fig. 92), if present, may be readily seen. Add a drop of concentrated sulphuric acid to the edge of the cover-glass, and observe closely the changes that take place. The crystals fade gradually, and their once rather distinct margins are changed to a yellowish or reddish color.

(*b*) Place crystals of cholesterin in a test-tube; add chloroform and a concentrated solution of sulphuric acid; shake vigorously for one-half minute. If cholesterin be present, the chloroform will be changed to a purplish-red or blood-red color. The layer of sulphuric acid becomes highly fluorescent.

MACROSCOPIC AND MICROSCOPIC CONSTITUENTS.

Particles of Food, etc.—Normal feces contain particles of undigested food and of solid materials that have been taken with the food. The network of vegetable cells is often seen, as well as distorted muscle-fibers of a faint yellow color and elastic fibers. The elastic fibers are of a clear, pearl-white shade, and even when slightly colored are distinguishable by the fact that they are well outlined and often present in apparent double contour. Starch-granules and small, flake-like particles of casein are also found in the normal feces. Muscle-fibers may be detected in every specimen of feces when the patient has been on a diet rich in meats. The number of these fibers present will depend upon the individual, the degree of mastication, the amount of meat ingested, and the thoroughness with which digestion has been accomplished.

It is not uncommon to find some muscle-fibers that have retained their characteristic outlines and others that show slight distortion; but most of the muscle tissue taken as food is so broken up that it cannot be recognized, or else is represented by small, disc-like, yellowish fragments. It is only after careful study that one is able to recognize these latter particles as partly digested muscle tissue. The most characteristic features of such particles are, first, their parallel outlines, and, second, their characteristic cross-striations, both of which features are best seen with a one-sixth or one-eighth objective (Fig. 142).

Clinical Significance.—A large number of connective-tissue fibers in the feces is conclusive evidence that digestion has not been completed, and is due either to a pathologic condition of the gastro-intestinal tract or to decided errors in diet.

Starch.—Starch-granules and chlorophyl are found in some of the undigested vegetable foods, but it is uncommon to find free starch-granules in normal feces, unless the patient has been fed upon food composed principally of starch. Starch-granules are, therefore, rather common in the feces of children who are fed upon prepared foods.

Under pathologic conditions it is not uncommon to find isolated starch-granules in the feces, as well as undigested particles of vegetable matter in which starch-cells are to be seen.

Detection.—A portion of the feces examined under the microscope should be treated with Lugol's iodin solution by placing a drop of this solution at the margin of the cover-glass, so that it gradually comes in contact with the specimen. Starch-granules, upon coming in contact with the iodin solution, change to blue.

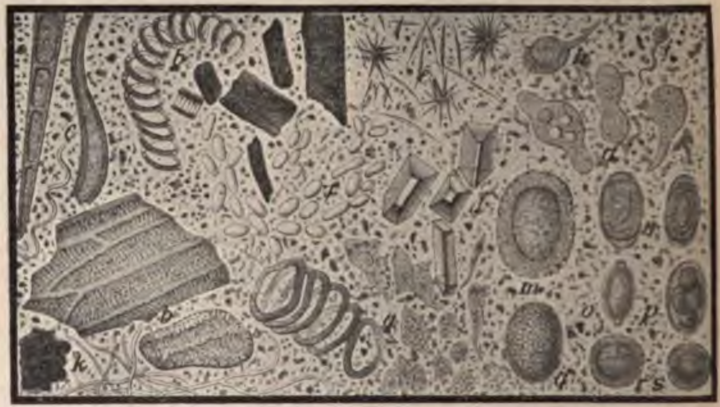

Fig. 142.—General view of the feces: *a*, Epithelial cells and leukocytes; *b*, stone-cells; *c*, cuticular formations; *d*, crystals of ammoniomagnesium phosphate; *e*, fat-crystals; *f*, yeast-fungi; *g*, Amœba coli; *h*, Trichomonas intestinalis; *i*, Cercomonas intestinalis; *m*, ovum of ascaris; *n*, ovum of oxyuris; *o*, ovum of trichocephalus; *p*, ovum of ankylostomum; *q*, ovum of bothriocephalus; *r*, ovum of Tænia saginata; *s*, ovum of Tænia solium (Jakob).

Mucoid, Serous, and Bloody Stools.—Mucus, when it is distinctly recognized in the stools, is indicative of a catarrhal inflammation of the mucous membrane of the intestine, yet this finding alone should not be regarded as one of serious moment, since it may occur in conjunction with but slight intestinal inflammation. There is normally a certain amount of mucus secreted by the intestine, and small particles of mucus may be seen clinging to the surface of firm stools. Large quantities and thick shreds or rope-like masses of mucus are suggestive of catarrh of the large intestine (Fig. 143), and in such a catarrhal condition rounded, sago-like granules of mucus are often found. Such bodies are seen in the rice-water stools of cholera; they are also found in the acute catarrhal conditions. Scybalous masses may also be coated with mucus, indicating a chronic intestinal catarrh.

The discharge of mucus is abundant in the feces in cases of acute intestinal catarrh whenever the large intestine is involved in the process, and in nearly all cases of catarrhal dysentery.

Microscopically, it is uncommon to find small particles of mucus in pathologic feces, but these may be present without carrying any clinical significance. Should such particles of mucus show evidence of bile-staining, they are indicative of catarrh of the small intestine. Numerous microscopic particles of unstained mucus are generally conceded to be suggestive of the existence of a catarrhal process high in the large intestine or in the lower section of the small intestine.

Caution.—Whenever the feces contain many small particles of mucus, it is well to test for starch by adding a drop of iodin solution.

Casts of the Bowel.—Mucus cylinders are at times passed with the dejecta. These may be mere shreds of mucus-like material or they may form a complete cast of the bowel. Such casts usually have a constant diameter throughout their entire length, and their free surfaces will be seen by the naked eye to be studded with fine white granules. When the inflammatory process is acute, their surfaces may be streaked with blood. Cylinders of the bowel may vary in length from two to sixteen inches in catarrhal enteritis. In the case of a woman studied in my clinic at the Howard Hospital, a cast from four to ten feet in length was passed at intervals of from ten days to two weeks. In the case of a child of three years recently under my observation, a mucous cast of the bowel was passed daily for a period of one week, each cast varying from ten to eighteen inches in length.

In cases of diphtheritic dysentery true casts of the bowel are passed with the feces. They are, as a rule, but a few inches in length, and are usually only partial casts of the intestine. Diphtheric casts of the bowel are much darker in color than mucous casts, and when studied microscopically, are seen to be formed of a true diphtheric membrane.

Microscopic Study.—Microscopically, these mucous casts are found to consist principally of a faintly opalescent, hazy, homogeneous material (mucus). A number of epithelial cells, leukocytes, red blood-cells, mucus-corpuscles, and Charcot-Leyden crystals appear to be entangled in this mucus-like substance. Other crystals are rarely seen. When the catarrhal process has gone on to the stage of necrosis of the epithelial cells lining the intestine, a true diphtheric membrane is formed which is much thicker than the mucous cast and contains necrotic material.

Clinical Significance.—Mucous casts of the bowel are found in connection with both chronic and acute inflammation of the intestinal mucosa. Their pathologic significance depends upon the numbers of epithelial cells which they contain, because the presence of these cells serves as a direct index of the degree of destruction that is taking place in the bowel. Mucous casts are not uncommon in gastroptosis and in enteroptosis.

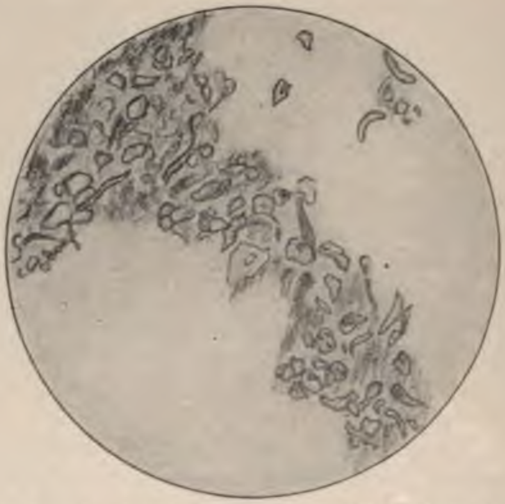

Fig. 143.—Portion of cast from bowel, observed at Howard Hospital (obj. Spencer one-sixth).

In primary diphtheric dysentery the passage of a large number of diphtheric casts with a considerable amount of blood is usually followed by death within the course of a few hours.

Secondary diphtheric dysentery, which usually develops late during the course of some chronic malady,—*e. g.*, diabetes, tuberculosis, etc.,—may persist over a long period of time, during which both long and short, rather pale casts of the bowel may be passed.

Epithelial Cells.—Under normal conditions but few epithelial cells are to be found in the feces, and, in fact, epithelial cells do not concern us from a practical standpoint except there be some inflammatory process of the intestinal mucosa.

It is not unusual, however, to find rather well-preserved cylindric (goblet) cells. Indeed, all the varieties of cells known to develop upon mucous surfaces are occasionally met with in the normal feces. When the epithelial cells have been separated from the mucous membrane for some time, they become distorted, probably because water is extracted from them.

The epithelial cells, when killed *en masse*, may be thrown off in the form of the bowel casts which have been previously described. In acute inflammatory processes the intestinal epithelial cells may appear in the feces in great numbers, a feature common to catarrhal dysentery. When the stools are decidedly watery, the epithelial cells appear rolled together, giving the liquid an appearance as though particles of cracked rice were floating in it. Again, other epithelial cells may be found more or less closely connected with shreds of mucus, and such cells at times show evidence of degeneration. Bile-stained epithelial cells are supposed to indicate a lesion of the small intestine. Cylindric and goblet-cells are not infrequently found in the dejecta of dysentery. Large clusters of epithelial cells have been reported as occurring in the feces of individuals suffering from carcinomatous disease of the rectum.

Blood-corpuscles.—It is unusual to find unaltered red blood-cells in the feces except when an ulceration involving either the lower portion of the colon or the rectum exists. In acute catarrhal dysentery mucobloody discharges are common, and in them the erythrocytes may be but slightly altered. It is to be borne in mind that the feces may be markedly colored with blood, and yet microscopic study will fail to reveal the presence of red blood-cells. It is impossible to recognize the red blood-cells as such in the feces when a hemorrhage has occurred high in the intestine, but instead of blood-cells, the feces will contain small, roundish, amorphous masses of a brown-red color which consist, in part at least, of hematoidin. Crystals of hematoidin are occasionally met with in the feces after intestinal hemorrhage (see Plate 17), but in such a condition crystalline hematoidin appears, for the most part, in the form of rhombic plates. In my own experience, I have been unable to find red corpuscles in the feces after a hemorrhage during the course of typhoid fever. In this condition the higher the seat of the hemorrhage, the more likely is one to find crystals of hematoidin and the darker in color will be the feces.

Leukocytes.—The appearance of a small number of leukocytes in the feces is of no practical clinical moment, but when leukocytes are present in abundance, they are suggestive of an inflammatory process, either catarrhal or ulcerative, which affects the intestinal mucous membrane. The number of leukocytes present in the feces is in direct relation to the degree and character of the lesion.

Pus.—Pure pus is seldom recovered from the feces; but when it is found, it indicates that an abscess has probably communicated with the bowel.

In acute catarrhal dysentery the feces are seen to contain blood, mucus, and a large quantity of pus. I have repeatedly seen mucopurulent and seropurulent stools in this disease, and it is not uncommon for the pus to form a large proportion of the entire stool, but it is extremely rare to find purulent stools devoid of serum, mucus, and blood. I have in mind the case of a woman, recently under observation, in which from one and one-half to three ounces of pure pus escaped with the daily stool. It is unusual to find blood in such stools, and they seldom contain mucus in appreciable amount. In conditions of this nature one is certainly not warranted in regarding such quantities of pus as being of intestinal origin. In the case just cited, for example, the clinical manifestations of catarrhal enteritis were wanting.

INTESTINAL CONCRETIONS.

Biliary.—The importance of the examination of the feces for the presence of biliary concretions is, doubtless, sufficiently emphasized by works in both medicine and surgery. It is a diagnostic method which should be employed whenever abdominal pains of an obscure or questionable nature or true hepatic colic exists.

Method of Detection.—(1) Collect every bowel movement for from twenty-four to forty-eight hours following an exacerbation of colic. (2) Direct that each stool be passed into a rather large commode. (3) Add to the stool one gallon of water, and stir thoroughly; allow the mixture to stand for a short time, and pour off the supernatant liquid. (4) Again add water, and stir to effect a perfect mixture. (5) Cut a piece of surgeon's gauze large enough to cover the top of a second vessel, and tie it to the rim of the top of the vessel with string or fasten with adhesive plaster.

The mixture of feces and water is now poured upon the gauze, which acts as a filter and separates any calculi that may be present. In this manner calculi may be readily detected. When found, they should be placed in a bottle and hermetically sealed, since nearly all gall-stones deteriorate upon exposure to air and light.

Biliary concretions vary in size from that of a grain of sand to that of a small olive. They are usually of a dark-yellowish, greenish, or black color. The composition of the calculi varies greatly: those, for example, consisting for the most part of biliary pigments will be brown in color, and will be found to sink in water. Chemically, such calculi may contain lime-salts and salts of copper and zinc. It is the rule to find calculi composed of calcium salts to be distorted and irregular in outline. Again, hepatic calculi may be composed exclusively of cholesterin, in which case they are soft and light in color, varying from white to a faint green shade. Cholesterin calculi are best distinguished by the fact that they are lighter than water.

It is reasonable to believe that every hepatic calculus consists of a nucleus, and this nucleus is often found to be composed of phosphates, and less frequently of sulphates. Joseph McFarland was the first one to recover a pure culture of the Bacillus coli communis from the nucleus of a gall-stone. Since his observation Welch and other observers have detected, in addition to the Bacillus coli communis, the Bacillus typhosus, in the nuclei of gall-stones. When it is desired that the exact composition of a gall-stone be ascertained, it will be found necessary to subject it to chemic analysis.

Intestinal Calculi.—It is exceptional to find intestinal concretions passed with the feces; but when such concretions are discovered in the stools, it is supposed they have been formed in the saccules of the colon or in the appendix. It is further supposed that the calculi result from the accumulation of calcium and magnesium salts upon solid particles which have probably been ingested and which have served as an appropriate nucleus for their development. Such calculi may form in the folds of the rectum, yet their usual seat of origin is in the cecum.

Intestinal Sand.—A singular case of intestinal sand was reported by Duckworth and Garrod,* who contribute an interesting paper upon the subject of true enteric lithiasis. I have been able to find reports of three other cases in the literature. The stools in the case cited by the above authors consisted of a variable amount of loose brown material which was found to contain about a teaspoonful of gritty sand. This sand was soluble in boiling nitric acid, but insoluble in cold liquor potassæ. It is of interest to note that the colon and the sigmoid flexure were readily palpable in Duckworth and Garrod's case. These authors suggest that there are true and false intestinal sands, the former consisting of organic material, the latter, of remnants of vegetable food.

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*"Lancet," March 8, 1902.

Dr. John K. Mitchell* has reported the case of a male, aged forty, from whom a quantity of intestinal sand was recovered. Dr. Mitchell tells me he has seen three additional cases where sand was passed with the feces. He further states that all these cases were upon a milk diet at the same time when such sand was passed.

Fatty Stools.—Under normal conditions the feces contain a variable amount of fat, either in the form of oil-droplets—highly refractile polygonal masses which vary in color from yellowish-white to dark yellow or red—or as needle-like crystals (see *Crystals of Feces*, below). Pathologic fat is usually recognized by the glistening, greasy appearance of the stool, which is often of a grayish-yellow color. When fat is present in large amounts, the feces are clay-colored and sometimes nearly white (it is not essential that the bile-duct be obstructed in order that the stools be white or clay-colored). Feces in which there is much fat become softer and develop a glistening appearance when slightly heated. A portion of fatty feces when placed under a one-sixth objective will show at least one, and probably all, of the above-named forms in which fat may appear. It is not infrequent, however, to find only the yellowish masses present, in which case add a drop of concentrated sulphuric acid to the margin of the cover-glass, remove the slide from the microscope, and heat it gently. This treatment will cause the masses of fat to break up and form oil-droplets. Whenever fat is found, it is stained black with a solution of osmic acid and pink with an alcoholic solution of Sudan III.

Clinical Significance.—Fat appears in the stools after the ingestion of large quantities of fat; during the course of diseases accompanied with progressive emaciation; and in the anemias, both primary and secondary. In such conditions the quantity of fat present in the stool is not large, as a rule. In all forms of obstructive jaundice the feces will be found to contain an abnormally large amount of fat. Fats are also found in the feces of persons suffering from pancreatic disease, and during the course of acute infectious fevers.

CRYSTALS OF THE FECES.

Crystals are among the commoner constituents of the feces, appearing in fairly large numbers under normal conditions. Certain crystalline bodies may be found in the feces of special maladies (see *Bloody and Fatty Stools*).

Fatty Acids.—Crystals of the fatty acids, when present in the feces, appear in the form of slender needles which are often arranged in rather dense aggregations, forming more or less perfect rosets (Fig. 97). These crystals are regarded by Gerhardt as consisting of calcium and magnesium salts of the higher fatty acids. Other observers have thought them to be crystals of tyrosin. Feces containing crystals of the higher fatty acids also contain crystals of calcium and magnesium soaps (Fig. 97), which have been described in the Chapter on *Urine* (page 266).

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* "Trans. Phila. Col. Physicians," 1903.

Clinical Significance.—Crystals of fatty acids, when abundant in the feces, point directly toward an impediment to the absorption of fats and also to some intestinal derangement. In all forms of obstructive jaundice crystals of the fatty acids are abundant in the feces. They are a normal constituent of the feces of children during the nursing period, and may be found in moderate amounts during childhood.

Charcot-Leyden Crystals.—Charcot-Leyden crystals (Fig. 181) may be found in normal stools and are to be seen in such febrile conditions as typhoid fever, dysentery, etc.

Following infection with a variety of intestinal parasites the feces may contain Charcot-Leyden crystals, as has been observed in connection with ankylostomiasis. They are found with less frequency in cases of tape-worm infection, and it has been stated that they do not occur in the feces during an infection with the dwarf tape-worm (Hymenolepis nana). They are also found with oxyuris, lumbricoid, and trichocephalus infections.

Charcot-Leyden crystals have been found in large numbers in the feces of amebic dysentery. My own experience has been that when the feces are sufficiently diluted, the finding of Charcot-Leyden crystals is the rule. When present in great numbers, they appear as dense aggregations.

Cholesterin.—Cholesterin is a normal constituent of the feces, although it usually appears in some form other than that of the characteristic plates (page 92).

Hematoidin.—Crystalline hematoidin appears in the feces whenever gastro-intestinal hemorrhage has occurred. The number of crystals present is usually in direct relation with the quantity of blood that has escaped into the bowel. Their number is also influenced by the site of the hemorrhage: the smallest number is found in the feces when the hemorrhage comes from the rectum (see *Bloody Stools*, page 372).

Phosphates.—The organic salts of lime are not infrequently found in the feces, and crystalline calcium phosphate may appear in dense clusters. Crystals of triple phosphate (ammonium, magnesium, and calcium) are also a normal constituent of the feces; they have been described in connection with the urine (page 275).

Bismuth.—Following the administration of large doses of bismuth subnitrate or of bismuth salicylate the feces may contain blackish crystals which slightly resemble crystals of hemin. These are crystals of bismuth sulphid.

BACTERIA.

All suspicious-looking particles should be removed from the feces by means of a needle and placed in a Petri dish, or probably a better procedure is to smear them thinly upon a slide, fix by heat, and stain. When the object is to study living parasites and to ascertain the general microscopic character of the feces, it is well to place the suspicious particle in the center of a slide, add one or more drops of water to it, and allow a cover-glass to fall gently upon the mixture. A specimen thus prepared may be studied under a one-sixth, one-eighth, or even an oil-immersion objective, so that the various bacteria and animal parasites, including amebæ, may be readily seen. The specimen often becomes chilled during the process of preparation, and then the Amœba coli, should it be present, would assume a spheric shape and become quiescent. Upon gently warming one extremity of the slide, however, the Amœba coli will assume decided motility, and may be seen retracting and throwing out pseudopodia in various directions. Motile bacteria also become active when thus warmed.

Care must be taken not to make the slide too hot while endeavoring to bring out the motility of organisms. A warm stage may be obtained for the microscope, and this will be found to prolong materially the life of the parasites found in the feces.

Tubercle Bacilli.—The detection of the tubercle bacillus in the feces is readily accomplished whenever ulceration of the intestine, due to the development of this organism, exists. Collect a small portion of the purulent or mucoid material from the feces, smear it thinly upon a glass slide, and stain it for the tubercle bacillus, as directed in the Chapter on *Sputum* (page 438). It is of special importance that when acid-resisting bacilli are found in the feces, they be carefully stained to differentiate between the tubercle bacillus and the other so-called acid-fast bacilli (page 441). When the feces have been allowed to stand for some hours, I have found that the condensation liquid that collects above the sediment of the feces often shows many tubercle bacilli; but this finding is by no means constant. Again, I have repeatedly examined the feces of persons suffering from tuberculous ulceration of the intestine in which it was impossible to find tubercle bacilli; yet these same individuals were found at postmortem to have extensive ulcerations of the intestines which were tuberculous in nature.

Clinical Significance.—Tubercle bacilli, when found in the feces, point conclusively to the existence of tuberculous ulceration of the intestines.

Pus-producing Organisms.—The feces always contain a great number of bacteria, the majority of which are non-pathogenic, but at times we find pus-producing organisms present in the feces, particularly in the dejecta of patients with acute dysentery or diphtheric dysentery. Streptococci, staphylococci, and bacilli are usually found in such cases. It is impossible to determine, merely from the staining properties of these organisms, which of them is of pathogenic importance. It, therefore, becomes necessary to make cultural studies of the bacteria found in the feces in cases of pathologic conditions of the intestines. Such cultural studies must be carried to the point at which the different organisms present are isolated. It is also necessary to make animal experiments in order to estimate the degree of pathogenicity possessed by any one organism recovered.

The typhoid bacillus may be recovered from the stools during the ulcerative stage of typhoid fever, but this method of study is more of scientific than of practical value, since the Widal reaction furnishes the most valuable clinical datum concerning this disease. The isolation of the Bacillus typhosus from the stools is a complicated and tedious process.

The **bacillus of Shiga** may be recovered from the dejecta of persons suffering from acute catarrhal dysentery, but this finding is much more constant in tropic and subtropic regions than it is in temperate climates. The bacillus of Shiga, when isolated from the feces of acute dysentery, will be found to agglutinate with the patient's serum (see *Serum-diagnosis*, page 116).

It is not within the scope of this volume to describe in detail the cultural characteristics of the various bacteria encountered in clinical diagnosis; but in view of the fact that bacillary dysentery has received special study by a number of investigators, among whom should be mentioned Krause, of Germany, Deycke, of Turkey, Froch von Drigalski and Conradi, of Prussia, and Flexner,* Duval, Vedder, and Mason, of the United States, and more recently by Duval and Basset, who have isolated this bacillus from the dejecta in summer diarrhea of infants, it appears warrantable to insert the morphologic and general cultural characteristics of Shiga's bacillus.

The bacillus of Shiga is a small, slender rod with somewhat rounded ends. Morphologically, it resembles the typhoid bacillus and the colon bacillus, to which group of bacilli it probably belongs. The bacillus is slightly motile, stains with the ordinary anilin dyes, and is decolorized by Gram's method. When specially cultivated and stained, it is furnished with flagella. The bacillus may be isolated from the feces during the first few days of the disease, but is not readily found during the subacute and chronic stages of dysentery.

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* "Phila. Med. Jour.," Sept. 1, 1900, p. 414.

"The value of bacillary diagnosis is lessened by the fact that the bacilli cannot often be found in the early stages or in mild cases of the disease, but when the morbid process is low down in the colon or in the rectum, they can generally be found without difficulty."* From the fifth to the seventh days of the disease the bacilli are abundant in the blood and mucus of the dejecta; but with the establishment of convalescence, the bacilli lessen in number and finally disappear, to reappear with the occurrence of relapses.

Isolation of Shiga's Bacillus.—The practical operation of isolating the bacillus of Shiga from the feces is as follows:

1. Tubes containing agar-agar are placed in a glass containing water that has been warmed to melt the medium. When the agar-agar is melted, it is inoculated with the mucous or the bloody portion of the stool.

2. The necessary care is to be employed, and the liquid agar-agar is poured into a Petri dish and placed in a cool place until the agar-agar has solidified. The dish is then placed in an incubator at a temperature of 37° C. (98.6° F.).

3. At the end of from twelve to twenty-four hours the dish is examined and cultures made from the isolated colonies of bacilli that have developed upon the surface of the agar-agar.

4. The cultures of bacilli and cocci are separated by the mere smearing of a slide with a portion of the colony, and staining to determine the morphology of the organism present. After determining which of the colonies contain bacilli only, the different forms of bacilli are then separated—"the practical operation of separating the different kinds of bacilli which grew in the plates was to inoculate glucose agar-agar stab-tubes from the different colonies" (Flexner).

5. Place these stab-cultures in the incubator at a temperature of 37° C. (98.6° F.) for twenty-four hours. At the end of this time those containing the colon bacillus and other gas-producing organisms will show evidence of gas-formation. Those tubes in which no gas has been evolved are probably inoculated with the

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* C. F. Mason, "Jour. Amer. Med. Ass.," July 23, 1903, p. 243.

bacillus of Shiga, and should be further studied to determine its identity.

Mason states that "if agglutinating reaction is positive" with the patient's blood-serum; if no gas is produced and milk is not coagulated, the micro-organism is the Bacillus dysenteriæ.

Shiga advances the following valid reasons for believing that this bacillus is the exciting cause of dysentery:

1. It may be cultivated from the feces in all cases.
2. It is not to be found in other disease or in health.
3. The number of bacilli present in the feces bears a direct relation to the intensity of the existing morbid process.
4. It is present in the lesions in the intestinal wall.
5. It agglutinates only with the blood of persons suffering from bacillary dysentery; and this agglutinative power is said to increase with the approach of and during convalescence.
6. A pure culture of the Bacillus dysenteriæ (Shiga) was intentionally given to a condemned criminal, who developed the disease. Infection also occurred after a pure culture had been accidentally drawn into the mouth. There are two other instances in which accidental infection occurred in man.

ANIMAL PARASITES OF THE FECES.

CLASSIFICATION.

I. PROTOZOA.

1. Rhizopoda	Zoölogists recognize six orders of this class of parasites, one of which is found in the human intestine and feces.
2. Ameba (Entamœba)	The Entamœba coli and E. histolytica (see page 388).
3. Flagellata s. mastigophora	Occasionally found in human feces (page 392).
4. Cercomonas (Lamblia, Megastoma)	Found in human feces (see page 394).
5. Trichomonas hominis	Found in human feces, less often in leukorrheal discharges, and in the urine (see pages 393, 484).
6. Balantidium coli	A parasite found in practically all parts of the Eastern Continent and in North America. Recovered from the feces of man and those of the swine (see page 395).
7. Trypanosoma	A genus of protozoa found in the blood of man and in the cerebrospinal fluid of persons suffering from sleeping-sickness. It is common in pus from abscesses of horses and in the blood of rats (see page 151).
8. Protozoön of Laveran (malarial parasite)	See *Malaria* (page 157).
9. Piroplasma hominis	A protozoön found in the blood of persons suffering from Rocky Mountain spotted fever (tick fever) (see page 155).

II. Entozoa.

1. Agamodistomum ophthalmobium (Fasciola ophthalmobium)	A rare parasite which invades the capsule of the crystalline lens. Probably identical with the liver fluke.
2. Ankylostoma (Uncinaria) duodenale and Americana	Infests the duodenum and small intestine; its ova are recovered from the feces (page 415).
3. Anguillula aceti	A parasite of the human urine (page 298).
4. Ascaris canis (or mystax)	A genus of nematoid intestinal worms (page 414); their ova are detected in the feces.
5. Ascaris lumbricoides	
6. Ascaris maritima	
7. Cysticercus cellulosæ (cysticercus of Tænia solium)	Genus of family of hydatids, and distinguished by the caudal vesicle in which the depressed body of the animal rests. Found in muscles, meninges, ventricles, and brain.
8. Cysticercus (C. tenuicollis) of Tænia marginata	A rare intestinal parasite.
9. Dibothriocephalus latus	The fish tape-worm, which is commonly referred to as Bothriocephalus latus (page 402).
10. Dicrocœlium lanceatum	A variety of liver fluke (page 412).
11. Diplogonoporus grandis	A rare parasite of the human intestine in Europe and North America.
12. Diplosoma crenatus	An entozoön four to eight inches long, which is bent upon itself at an acute angle. Habitat, the urinary bladder. May be passed with the urine.
13. Dipylidium (Tænia) caninum	Dog tape-worm, rarely found in the intestine of man (page 407).
14. Dracunculus medinensis	Guinea-worm or thread-worm. Invades the areolar tissue and skin of the legs and feet (page 140).
15. Echinococcus polymorphus (hydatid of Tænia echinococcus)	Liver, spleen, lung, brain, meninges, and omentum. Larval form of the dog tape-worm, and develops in man as a hydatid cyst (see also No. 8).
16. Eimeria bigemina	Invades the intestinal epithelium of man and of animals (dog and sheep).
17. Eustrongylus gigas	The largest of this genus. Found in the human kidney and intestine; also in the kidney of the lower animals.
18. Fasciolopsis buskii	A trematode worm found in the gall-bladder and duodenum of residents of Asia.
19. Fasciola hæmatobium	Same as No. 34.
20. Fasciola hepatica	The common liver fluke (page 411).
21. Fasciola hepatica Ægyptiaca	The Egyptian liver fluke (page 412).
22. Fasciola hepatica angusta	The narrow liver fluke (page 412).
23. Filaria medinensis	See No. 14.
24. Filaria oculi or loa	May invade the eye, but it is also allied to Filaria perstans (page 147).
25. Filaria sanguinis hominis	Infests blood of man, animals, and birds. Species of filaria are common in crows, blackbirds, wild ducks, and the porcupine (page 147).

26. Heterophyes heterophyes	Inhabits the small intestine.
27. Monostoma lentis	See No. 20.
28. Œstrus hominis	A bot-fly which deposits its ova in the skin; also in the external auditory canal (page 470).
29. Opisthorcis noverca	Trematode worms found in the gall-bladder and duodenum of residents of Asia.
30. Opisthorcis sinensis	
31. Oxyuris vermicularis	A nematoid worm—pin-worm, seat-worm—infesting the rectum and vagina (page 414).
32. Paragonimus Westermanii	A parasite of the lungs, liver, and connective tissue (page 435).
33. Pentastoma constricta (Porocephalus constrictus)	A genus of entozoa parasitic to man and animals. A long, cylindric, annulated worm infesting the intestine; it may become encysted in the liver and other tissues.
34. Pentastoma denticulatum (Linguatula rhinaria).	
35. Schistosoma (Bilharzia) hæmatobium	A genus of trematoid worms that inhabit the portal, bladder, hemorrhoidal, and pulmonary veins; its ova are to be found in the urine, feces, and sputum (pages 295, 436).
35A. Schistosoma Cattoi	Resembles 35 except the ova escape into bowel and the worm invades arteries instead of veins.
36. Strongyloides stercoralis (intestinalis	Cause of Cochin-China diarrhea (page 419).
37. Strongylus bronchialis	A nematode worm from the human bronchus.
38. Strongylus gigas (Ascaris renalis)	See No. 16.
39. Strongylus subtilis	Japanese diarrhea worm (page 421).
Tænia acanthotrias	A rare finding in man.
40. Tænia africana	See page 409.
41. Tænia echinococcus	See No. 15; also page 404.
42. Tænia elliptica	See same as 13.
43. Tænia flavopunctata (Hymenolepis diminuta)	A parasite rarely found in the intestine of man; its ova closely resemble the ova of Tænia solium.
44. Tænia (Davainea) madagascariensis	See page 409.
45. Tænia marginata	The dog and sheep tape-worm (page 406).
46. Tænia mediocanellata	The beef tape-worm (page 399).
47. Tænia (Hymenolepis) nana	Dwarf tape-worm which invades the intestine of man and the lower animals (page 406).
48. Tænia solium	Pork tape-worm. A rare American parasite which inhabits the small intestine of man (page 398); also No. 7.
49. Trichinella (Trichina) spiralis	The adult worm invades the intestine of man and of the lower animals, and is taken into the stomach through infected pork. The larval form of the worm is deposited in the intestinal wall, and from there migrates to all parts of the body. These larva become encysted in the muscles (page 422).
50. Trichocephalus dispar (Trichuris trichuria)	Found in the cecum and colon of man, and is associated with the ankylostoma. The ova are detected in the feces (page 418).

III. Ectozoa.

1. Acarus (Demodex) folliculorum	Invades the sebaceous follicles of the skin, and also the conjunctiva.
2. Pulex (Sarcopsylla) penetrans (chigo)	A variety of tick found in Brazil and tropic districts. It invades the skin and cellular tissues.

GROSS EXAMINATION.

Of the various methods employed for the diagnosis of intestinal parasites, a microscopic study of the feces is most reliable. There are other methods of study, however, which should not be neglected. It is possible, at least, to suspect the presence of intestinal parasites from an examination of the blood, since eosinophilia is often associated with such infections.

A gross examination of the feces is of equal importance in the adult and in the child; in some cases such an examination may reveal segments of a tape-worm (Fig. 151); in the case of nematode infection the adult worm may be seen. Adult round-worms are likely to appear in the feces after the administration of anthelmintics, and are at times found after the patient has taken a liberal dose of calomel. During the first stage of trichinosis, when there is an associated diarrhea, the adult trichinella may be found in the feces (Fig. 173). The uncinaria and the oxyuris may also be detected in the diarrheal discharges. The technic for the detection of these parasites differs in no way from that outlined for the detection of the head of the tape-worm (page 397).

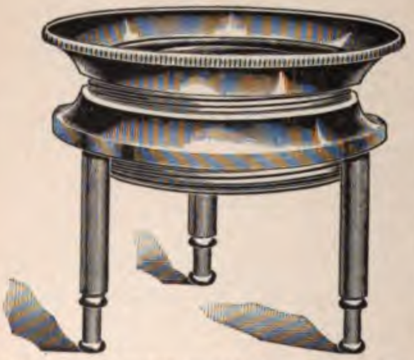

Fig. 144.—Magnifier mounted on tripod, for gross examination of feces for parasites.

When there is severe hook-worm infection, the color of the stools is often reddish brown. Ashford states that this color is probably due to the presence of blood in the stools, and that the blood reaches the intestinal canal from the openings made in the intestinal wall by the biting of the parasites. He further suggests that the anemia produced by such an infection is in part due to the continuous oozing of blood from the intestinal mucosa.

Blotting-paper Test.—Place several ounces of the fresh stool on a white filter-paper, wrap the paper tightly around the feces, and allow it to stand for several hours; then unwrap the package and examine the paper. In severe and sometimes in mild cases of hook-worm infection a distinct reddish-brown stain is present. This test is reliable in 70 per cent. of cases (Stiles).

Microscopic Study.—The microscopic characteristics of the feces have been discussed in connection with each intestinal parasite, but in a general way it may be well to consider a few points of practical interest. In collecting feces for microscopic study 1 to 5 gm. of the stool may either be wrapped in a piece of paper or placed in a wide-mouthed bottle and sent to the laboratory. It is better to prevent the stool from drying until the examination is completed, because while an expert microscopist may examine a stool that is several days old, far more satisfactory results are to be obtained from the study of fresh specimens.

Washing of the Feces.—Take one or more ounces of either the fresh or the dried feces, add one to two quarts of water, and stir until the feces are well mixed; allow to stand for a time to permit of sedimentation. Pour off the floating matter and the water down to near the sediment; repeat this washing several times and until no material floats upon the surface after sedimentation. Place the washed sediment in a conic glass and allow it to stand for a few hours, when it will be found to contain ova of whatever parasites may be present in the intestinal canal. Washing of the feces is not necessary for the detection of the Amœba.

Apparatus.—The apparatus necessary for microscopic examination of the feces includes a microscope, glass slides (two by three inches), large square cover-glasses, and a platinum needle, or, what is more commonly employed, a match-stick (see *Detection of Ova of the Uncinaria*, page 417). At least ten slides should be examined before a negative diagnosis is given.

Cautions.—It is of utmost importance that the feces be kept in a bottle that is well corked in order that the specimen be protected from flies. The greatest care should be exercised to prevent soiling of the hands during the examination, and all glassware should be placed in boiling water after use. It is well for the operator to remember that the feces of persons infected with tape-worm are highly dangerous to man.

A microscopic study of the feces is of no value unless one is familiar with the appearances of the eggs and embryos of the different animal parasites (see Plates 26, 27). All suspected objects should be studied most carefully, since plant-cells and fibers which closely resemble both the ova and the embryos of intestinal worms are always present in the feces.

The ova of animal parasites may appear in the feces when the adult parasite resides elsewhere than in the intestinal canal. The ordinary liver-fluke (page 411); the paragonimus (page 435), which invade the lungs, and the Schistosoma hæmatobium, which invades the vesical veins (page 295), are examples. It is, therefore, necessary that the sputum and the urine be studied conjointly with the feces in the diagnosis of animal parasites.

INTESTINAL COCCIDIOSIS.

It has long been known that coccidia were liable to infest the intestinal wall of domestic animals. Stiles has recently reported an interesting example of intestinal coccidiosis occurring in the sheep.* The Coccidium bigeminum (Eimeria bigemina) will be found to vary in length from 0.008 to 0.015 mm. According to Stiles, the size of this parasite varies, depending upon the animal (host) in which it has developed. Intestinal coccidiosis may develop in man. The accompanying illustrations (Plate 24) will serve to explain the peculiar features of this parasite, as well as to describe the slight differences between the Coccidium bigeminum and other coccidia.

RHIZOPODA.

The feature characteristic of this class of organisms is that its movements are caused by the throwing-out of prolongations, or *pseudopods*, from its body. These pseudopods result from protoplasmic activity, which enables the cell to protrude a pseudopod from any portion of its body. Rhizopoda are occasionally met with in the human feces.

Amœba Coli.—The member of the above group of animal parasites which concerns us most as clinicians is the Amœba coli (Lösch), which was first described in 1875. Lösch, at this date, detected for the first time the Amœba coli in the feces of persons suffering from tropic dysentery. At present the Amœba coli has been found in the feces of dysenteric patients in practically all parts of the civilized world. Amebic dysentery has received special study in India, China, Japan, and in European and American tropic districts.

Cases have been transported from the tropics to all portions of the world, yet the disease seldom, if ever, develops in temperate climates.

I have studied both sputum and feces in which amebæ were

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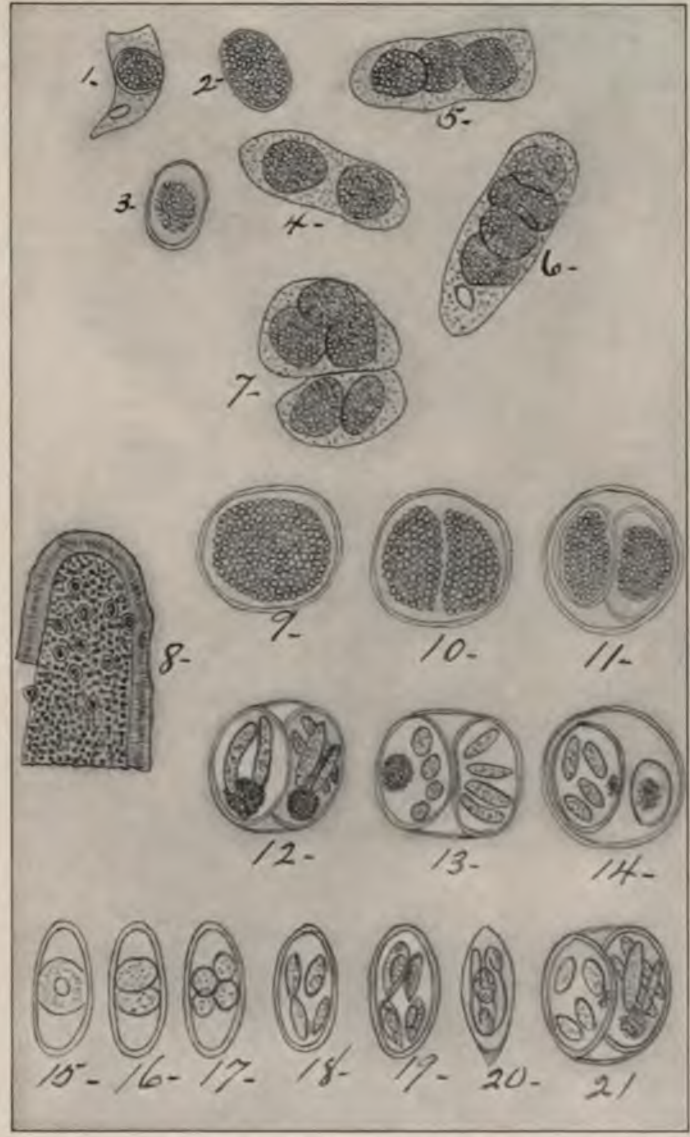
* "Jour. Comp. Med." and "Vet. Arch.," vol. xiii No. 5, pp. 319, 325.

PLATE 24.



1–7. Sporozoa (probably Coccidium perforans) from the intestinal epithelium of a sheep: 1, Epithelial cell containing single parasite; 2, free parasite; 3, the plasma has receded from cyst-wall; 5–7, two to four parasites in each cell (Stiles).

8. Portions of a villus of dog's intestine, showing eleven Coccidia bigemini.

9–14. Various stages in the development of Coccidium bigeminum (highly magnified) (Stiles).

15–20. Coccidium oviforme of rabbit (Stiles, from Balbiani): 15 corresponds to 3; 16, plasma divided into two sporoblasts; 17, further division; 18, sporoblasts are elongated; 19, they have become spores.

21. Coccidium bigeminum to show four sporoblasts and rest of segmentation (Stiles).

present (Fig. 145). The patients from whom the specimens were obtained had previously resided in the Philippine Islands. Cunningham has found that the ameba is fairly common in the stools of cholera patients. Grassi and Massiotin report having found amebæ in normal feces. George Dock, of Ann Arbor, has also detected amebæ, indistinguishable from the Amœba coli, in normal feces.

The fact that amebæ have been found in normal feces and in feces of diseases other than dysentery has led to the supposition that there are two species of Amœba—the *Amœba (Entamœba) coli*, which is harmless, and the *Amœba (Entamœba) histolytica*, which is concerned in the etiology of tropical dysentery, and of tropical abscess of the liver and lungs. A case of the latter condition has been studied by the author.*

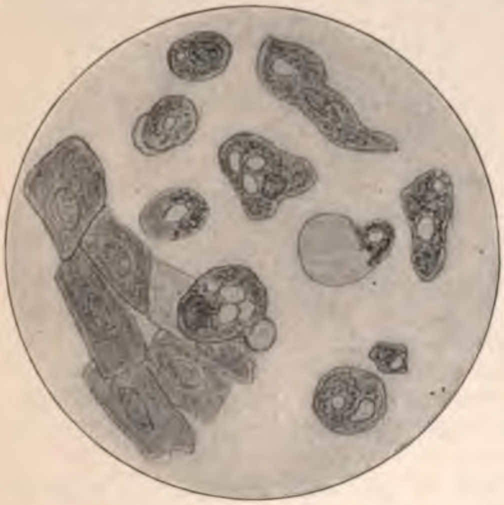


Fig. 145.—Amœba coli and cluster of epithelial cells from the sputum; private patient (obj. B. and L. one-twelfth oil-immersion).

Collection of the Feces.—In the study of feces for the detection of the Amœbæ the precautions already described on page 356 should be taken in the collection of the specimen.

The rectal mucus is most likely to contain the amebæ, and is best collected by introducing a large rubber catheter into the rectum. It is my practice to cut several rather roughly outlined openings in the side of the catheter. Immerse such a catheter in warm water, and introduce it into the rectum for a distance of from three to six inches or more. The mucus, blood, or pus that may cling to the catheter is most likely to contain the amebæ. This material is equally valuable in searching for the ova of practically all intestinal parasites. The administration of a vermicide does not appear warrantable except after positive results are obtained from a study of the rectal mucus, blood, or pus.

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* "Proc. Phila. Co. Med. Soc.," Sept., 1902.

Detection of the Parasites.—Place a small drop of the liquid portion of the feces or of the mucus, blood, or pus upon the center of a slide, add a drop of distilled water, mix the specimen with the water, and allow a cover-glass to fall gently upon the mixture. Warm one end of the slide slightly and then examine the specimen with a one-sixth or a one-eighth objective. With the magnification thus obtained it is possible to detect the ameba, and then further study may be made by bringing individual organisms into the field of an oil-immersion objective.

The Amœba coli is a unicellular animal parasite which varies from 13 to 37 μ in diameter.* Whenever the feces or liquid containing the Amœba coli is allowed to become cold, the amebæ at once assume a more or less spheric form and show no evidence of movement. During this quiescent state they are found to vary greatly in size; their surface is in part granular, while the remaining portion is hyaline, although the entire organism may display a more or less granular surface. It is impossible to detect the Amœba coli in the feces when the dejecta is at room-temperature, but when the slide containing the feces is placed upon a warm stage or heated gently over a flame and studied under a one-twelfth oil-immersion objective, the spheric granular bodies present in the cold specimen will be found to have assumed irregular shapes.

By watching an individual ameba it will be seen to throw out distinct prolongations (pseudopods) of its protoplasm. The projected portion of the cells is at first a mere bulging of the cell-wall; it then becomes oval, and finally appears like a pedicle. The pseudopod does not contain any of the granular protoplasm. After the cell-wall has been protruded for some distance, a protoplasmic current may be seen extending into this protrusion, and the granular protoplasm of the cell flows from its former resting-place to this protruded part of the cell. By the time all the granules have completely changed their position the cell will, in all probability, have become more or less spheric in outline—an apparent retraction of the protruded portion of the cell-body.

These movements are followed by a projection of the protoplasm from another portion of the cell-body, when the granules again change their position.

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* Dock, "Daniels' Texas Med. Jour.," 1891.

Caution.—It is a very easy matter for the inexperienced to mistake epithelioid cells, which are often present in the feces, for the Amœba coli. These epithelioid cells display a violent protoplasmic motion, and there is a slight movement of the cell's margin, but true pseudopods are not formed.

In all active amebæ a variable number of hyaline areas are to be seen (vacuoles). These vacuoles, as a rule, number from two to four, and are highly conspicuous in the motile parasite. They may also be detected in the non-active amebæ. The vacuoles may be situated within the granular portion of the parasite, and it is quite common to find the protoplasm more granular around the vacuoles than at other portions. At times, immediately surrounding the vacuole, there is a series of clear vesicles which are nearly uniform in size. These vesicles may occur at any portion of the body, and are more pronounced in some organisms than in others. During the ameboid movements of the parasites both the vacuoles and the vesicles appear to change in size.

Nucleus.—The nucleus of the Amœba coli is fairly characteristic, although it is much less conspicuous than are the vacuoles. The nucleus is best seen when there is a low degree of illumination; in fact, amebæ are best studied with a small amount of light. The nucleus of the Amœba coli will be found quite distinct in certain parasites (Fig. 145), while in others it is detected only after the most careful microscopic study.

I have endeavored, in the accompanying illustration (Fig. 145), to set forth the peculiarities known to the Amœba coli. These features are in a great measure common to all amebæ.

Life-cycle.—The life-cycle of the Amœba coli within the human body is a subject which has received much research, and until quite recently but little definite knowledge has been obtained. A knowledge of the methods through which other similar animal parasites develop has served to direct various investigators, among whom Grassi and Doflein and also Clarke * have considered the development of the Amœba coli in comparison with that of the malarial parasite. Schaudin proved that nuclear division is not constant and regular in the "Amœba coli," but, instead of this, a fragmentation of the chromatin takes place, "the remnant of the nucleus being expelled from the parasite." This division of the chromatin is followed by the formation of small spheric bodies, each of which is thought to contain a portion of chromatin. Schaudin further showed that these spores were capable of producing intestinal symptoms and lesions. The above peculiarities are not known to be common to other amebæ found in the intestinal canal. More recently Surgeon Charles Craig * in a preliminary note, in addition to making reference to the study of 748 cases of amebic dysentery, has considered the development of the ameba.

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* "Protozoa and Disease," p. 36.

Blood-cells.—Individual amebæ are at times seen which have engulfed one or more red blood-cells, leukocytes, bacteria, and granular detritus. The red cell is often situated near the center of the ameba, and may be completely surrounded; in fact, it is sometimes partially obscured by the granular protoplasm.

Stain.—The Amœba coli stains with the ordinary anilin dyes (a 2 per cent. aqueous solution of methylene-blue) and carbolfuchsin. It is with some difficulty that the ameba is well stained when in the feces; but the parasite is readily stained when in the sputum or in pus from amebic abscesses. When stained with methylene-blue and fuchsin, full-grown amebæ display from one to three vacuoles which are unstained. There are also to be seen numerous small, round or oval, dimly stained areas which are rather regularly disseminated throughout the protoplasm.

Clinical Significance.—Amœbæ coli may be found in the stools during the course of amebic dysentery. They are often present in the fluid obtained from the so-called amebic abscess of the liver, and from the sputum in cases where hepatic abscess has ruptured into the lung (see *Sputum*, page 436).

FLAGELLATA.

Members of the group flagellata or mastigophora are characterized by the fact that each organism displays from one to eight flagella. These flagella, by their active movements, render the animal capable of locomotion. It is not uncommon to find members of this class of parasites in the feces.

Cercomonadina.—The parasites of this family are small, and may be either oval or slightly elongated in outline. They are provided with one rather long flagellum at their anterior extremity, and contain one or more vacuoles. These parasites are also capable of ameboid movement, and may be seen to protrude pseudopods from their posterior extremity. The cercomonas described by Davaine and Lambl is a representative of this family of parasites (page 394).

Tetramitina.—Another parasite, the tetramitina, is a small, elongated body which possesses an undulating membrane and four distinct flagella. This parasite tapers gradually at one extremity and has a distinct nucleus. Tetramitina may be detected in the feces, in the vaginal discharges, and in the urine. They are probably identical with the trichomonas and cercomonas seen in these situations (Fig. 146).

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* "Amer. Med.," Feb. 20, 1904.

Trichomonas.—This parasite has received a variety of names, many of which refer to the situation from which it was recovered, while others refer to its peculiar biologic characteristics. The Trichomonas intestinalis is an animal parasite (Fig. 146) which may be oval or spindle-shaped, and which varies in size from 0.012 to 0.03 mm. by 0.01 to 0.015 mm.

The trichomonas is provided with an undulating membrane which extends from the parasite as a tail-like projection. Four flagella of varying sizes project from the anterior pole of the organism. It is very difficult to detect either the undulating membrane or the flagella when the animal is in motion. When a given liquid is known to contain the trichomonas, it is well to add a solution of mercuric chlorid (1–6000) to it. After this treatment both the flagella and the nucleus of the parasite become slightly more conspicuous. Specimens thus treated will be found to stain with the ordinary anilin dyes, and under these conditions the nucleus may be clearly outlined. The trichomonas is said to possess the faculty of ameboid movements.

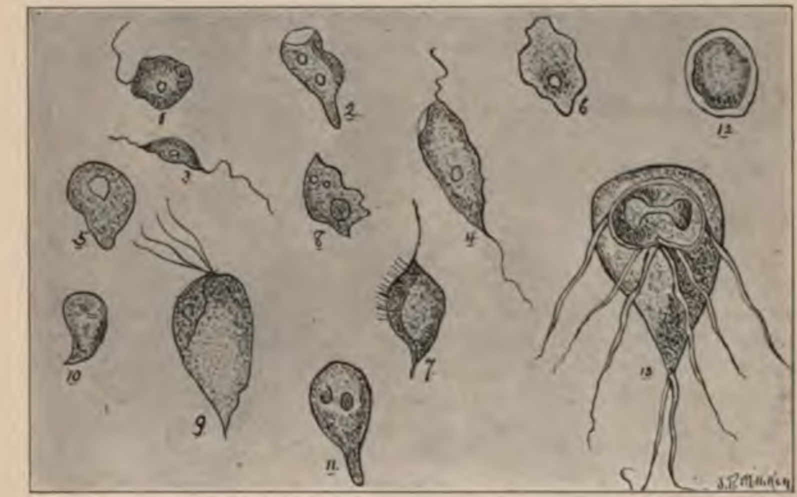

Fig. 146.—1, 2, 3, 4, 5, 10, and 11, Various forms of Cercomonas intestinalis (after Leuckart and Lambl). 6, 7, 8, and 9, Various forms of trichomonas (after Scanzoni and Kölliker and Dock). 12, Encysted form, and 13, adult form, of Megastoma entericum (Cercomonas) (after Grassi and Schewiakoff).

In the study of Philadelphia water I found that by the additio of a small amount of hay, for the purpose of developing certai bacteria, many small organisms which were apparently identica with the trichomonas were invariably found after the hay an water had been allowed to stand at room-temperature for a few days.

The technic necessary for the detection of the trichomonas is essentially the same as has been described for the detection of the Amœba coli (page 390).

Cercomonas intestinalis (Lamblia duodenalis).—Grassi has described this parasite under the name of Megastoma entericum (Fig. 146). Davaine refers to this parasite as the Cercomonas hominis, and many other writers have given this organism names more or less expressive of the location in which it has been found, or of its characteristic peculiarity. It does not appear practical to me to endeavor in this volume to set forth the distinctive differences known to exist between species of the cercomonas obtained from the various sources.

Generally speaking, the cercomonas is pear-shaped, and varies in length from 0.01 to 0.21 mm. In breadth the cercomonas varies between 0.0075 and 0.05 mm. The longer forms of this parasite are sometimes more or less S shaped. At the anterior portion of the cercomonas there is a fairly well-defined indentation, which is the peristome or mouth of the animal. The cercomonas, as a rule, has eight flagella, which project from the body in pairs. It is impossible to see these flagella in the actively motile parasite, but they are readily seen after treatment with a 1–5000 solution of mercuric chlorid. It is to be noticed that the flagella of the cercomonas are directed backward (Fig. 146). The first pair are given off from the sides of the anterior opening of the parasite, and two additional pairs of flagella are given off just posterior to this depression. The fourth pair of flagella project from the tail. It is often very difficult, even in the well-prepared specimen, to detect the eight flagella and, in fact, it is the rule to find many of these flagella twisted or plaited together to form a rather large flagellum.

This organism, when in its quiescent state, may assume an oval, elliptic, or spheric shape, and its flagella may not be apparent. For this reason and for many others my experience has indicated that it is not at all times possible to distinguish the cercomonas from other similar animal parasites.

Clinical Significance.—The cercomonas may be found in the feces of man and the lower animals. It is rarely encountered in the vaginal secretions and in the urine. Its pathogenic power is questioned.

Balantidium Coli.—The Balantidium coli (Paramœcium coli) is an oval organism which measures about 1 mm. in its greatest diameter (Figs. 147, 148). This organism differs from the organisms just described in that its entire body is covered with fine cilia. These cilia are thickest surrounding the mouth of the parasite, and are thinly distributed over the body and around the anus. The Balantidium coli has a pale nucleus and from two to four vesicles.

Within the body of the parasite small particles of starch may be seen, and at times droplets of fat are detected.

Clinical Significance.—Infection with the Balantidium coli is supposed to take place from the dejecta of swine. This parasite has been known to affect man in various portions of Europe, Asia, and North America. Ehrenroth,* in addition to reporting a fatal case of dysentery due to infection with the Balantidium coli, describes interesting pathologic changes present in the stomach and intestine (ulcerations). All together 89 cases of persistent diarrhea due to this parasite have been reported. It is of special interest to note that the profound anemia caused by the Balantidium coli resembles closely that present in the later stages of gastric carcinoma.

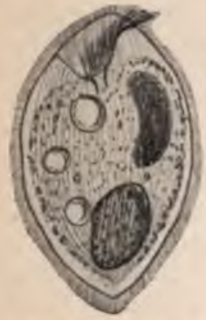

Fig. 147.—Balantidium coli (after Malmsten).

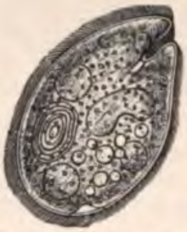

Fig. 148.—Balantidium (Paramœcium) coli (Eichhorst).

The Balantidium coli has been found in the feces of persons suffering from infection with the Dibothriocephalus latus; and it has been suggested that other intestinal parasites render the gastro-intestinal canal in a receptive state for infection with the Balantidium coli. In the fatal cases ulcerative colitis appears to be regarded as the cause of death.

Detection.—The technic necessary to detect the Balantidium coli in the feces differs in no way from that described for the detection of the Amœba.

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* "Zeit. f. klin. Med.," vol. xlix, p. 251.

TAPE-WORMS (CESTODES).

The habitat of this variety of parasites is the small intestine, where they give rise to considerable irritation, which results in a variable amount of intestinal catarrh. As the result of the intestinal catarrh, body depletion, toxemia, decided nervous manifestations, and severe anemia may follow infection with such parasites.

In reference to tape-worms in general it may be said that the head of the parasite is extremely small, and in some of the species resembles the head of a black pin. The head of the tape-worm may be armed with a complete crown of hooklets, and may also possess deeply pigmented or colorless depressions, which serve as sucking cups (Figs. 150, 158). The segments (proglottides) of the tape-worm always vary greatly in size (Fig. 151)—the longer the tape-worm, that is, the farther from the head, the larger is the segment. Extending from the largest of the segments toward the head, it is immediately apparent that each segment lessens in size until the neighborhood of the head of the parasite is reached, where the segments appear to the naked eye as a slightly flattened thread. The segments of the tape-worm are of a yellowish-white or bluish-white color.

The technic for the recognition of animal parasites in the human feces is of special importance in view of the fact that there is no other secretion or excretion wherein the offending object is surrounded by so large an amount of débris. It is fairly easy, however, to detect segments of tape-worms and the adult forms of other parasites when present in the feces. The search for the head of a tape-worm in the feces, after the administration of vermicidal drugs, is of paramount importance in the study of the parasite.

Removal of Tape-worms.—Before attempting to remove a tape-worm from the intestinal canal the physician should be sure that the patient's stomach is entirely empty, and that since his last meal sufficient magnesium citrate solution has been administered to produce two or more copious bowel movements, so that the greater part of the intestinal mucus has been expelled. It is the habit of the tape-worm to bury its head in the folds of the intestinal mucous membrane (valvulæ conniventes), and mucus collects about the point at which the worm is anchored to the intestinal wall. Unless this fortification of mucus be removed before the administration of a vermicide, it is improbable that the drug can reach the head of the parasite.

To Detect the Head.—The majority of failures in the treatment of tape-worms is due to the inability of the host to expel the head of the parasite, and since this is most important, I recommend the following routine procedure for the accomplishment of the object: (1) Empty the bowels by means of salines, so that no undigested food remains in the alimentary tract, and to insure that the head of the worm is uncovered by the removal of all intestinal mucus. (2) Administer a vermicide. (3) Follow in from four to six hours by another saline. (4) When the worm begins to escape from the anus, direct the patient to take a comfortable seat, so that the parasite may be received in a clean vessel containing water. (5) It is all-important that the patient should sit on one commode from the time he observes that the worm is diminishing in size until the entire parasite is passed (*the nearer the head, the smaller are the segments*). When within a few inches—ten to twelve—of the head, the worm appears as a pale, slightly flattened thread, and its segments are not distinct. (6) The head is the last portion of the worm to be passed. While any part of the parasite is protruding from the rectum the probabilities are that the head has not yet escaped.

Given a specimen collected in this manner, add a quantity of water and stir gently with a glass rod, so that the worm will fall to the bottom of the vessel. Decant one-half or more of the liquid, and replace it with clean water; repeat this washing until the worm is cleansed. Transfer the worm with some of the water to a clear glass dish 10 by 12 by 3 inches (Fig. 149), and place the dish on a white surface (towel). Remove all the large segments of the parasite by a glass rod, drawing them over the edge of the first dish and allowing them to fall into a second dish containing water, taking care not to break the parasite.

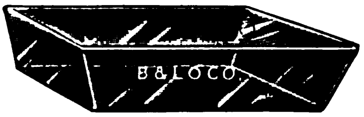


Fig. 149.—Dish for study of feces.

After all large segments are removed the head may, as a rule, be readily detected by the naked eye floating among the remaining thread-like portions of the parasite. In searching for certain small parasites a hand-glass is found of service. The head should be transferred to a 10 per cent. glycerin solution and preserved for further study. In mounting the head of a parasite a slide provided with a concavity of sufficient depth to accommodate its thickest portion is most satisfactory. The specimen may be preserved by mounting it in cast medium, Farrant's medium, glycerin, or glycerin-jelly.

Tænia Solium.—This parasite, the pork tape-worm, is fairly common in Europe, Asia, and Africa, but it is rarely found in America; and whenever found in this country, the question of the patient's having been abroad should be thoroughly investigated. The Tænia solium when in the human intestine may vary greatly in length, but this is of minor importance, since the length of any tape-worm does not appear to influence materially the symptoms resulting from infection with such a parasite (see *Method for Finding Head*, page 397).

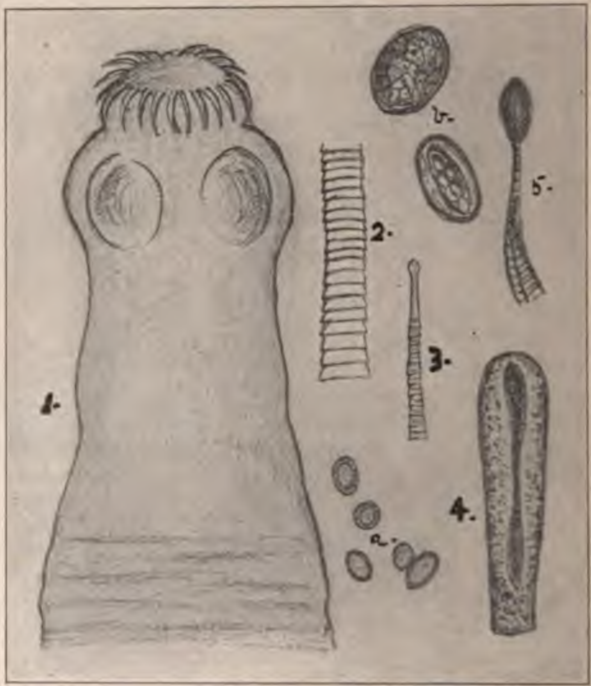
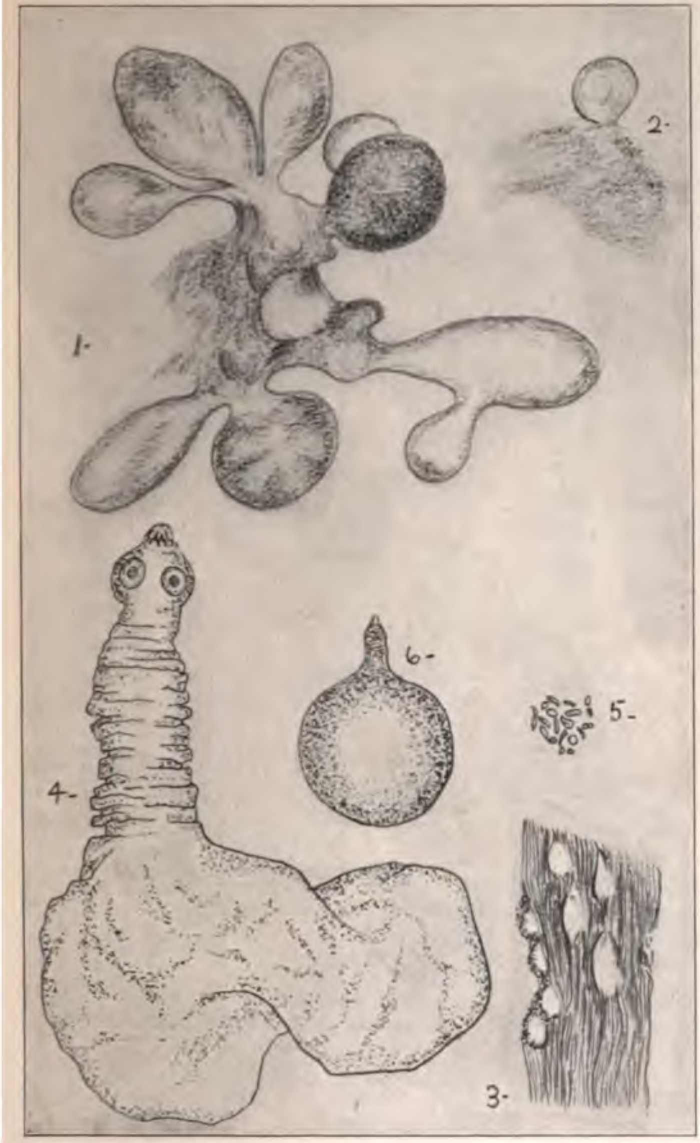


Fig. 150.—Tænia solium: 1, Head (obj. B. and L. two-thirds); 2, segments; 3, head and neck (natural size); *a*, ova (obj. B. and L. one-sixth). 4, Head of Dibothriocephalus latus (magnified). 5, Dibothriocephalus latus (natural size); *b*, ova (magnified).

The head of the Tænia solium (Fig. 150) is provided with four pigmented sucking-cups, which are best seen when examined with a two-thirds objective. It is usually possible to outline clearly a complete crown of hooklets at the tip of the head with a one-fourth objective. The fully matured proglottides (segments) are about 10 mm. long by 3 to 5 mm. wide (Fig. 151). The matured proglottides of the pork tape-worm present certain features which

PLATE 25.



1. Cluster of cysts (Cysticercus cellulosæ) recovered from lateral ventricle of in in Lloyd's case (original).
2. Cyst found in fourth ventricle (original).
3. A piece of pork infested with pork-measles (Cysticercus cellulosæ) (Stiles).
4. Isolated pork-measle bladder-worm (Cysticercus cellulosæ), with extended d, greatly enlarged (Stiles).
5. Young cysticerci (Cysticercus tenuicollis) of the Tænia marginata (Curtice).
6. Cysticercus tenuicollis with head extended from body, from a steer (Stiles).

serve to distinguish them from those of the beef tape-worm, so that by a study of these segments a diagnosis of the species present in the intestine may be made. Segments of the Tænia solium have a longitudinal canal or uterus from which several branches (eight to fourteen) extend laterally.

The ova of the Tænia solium are secreted constantly, and appear in the feces in large numbers. They are round and, when fully developed, are furnished with a shell upon which it is possible, by carefully focusing the microscope (one-sixth objective), to distinguish fine radiating lines. Firm pressure upon the cover-glass usually results in rupturing the operculum (shell), which often flies into many pieces.

When ova of the Tænia solium enter the human stomach from the mouth or are regurgitated from the intestine, the parasite develops only to its larval stage. Each larva subsequently becomes encysted in the human tissues or viscera, where it is known as the Cysticercus cellulosæ (Plate 25).

Tænia Mediocanellata.—The beef tape-worm (Tænia saginata) is found in nearly all parts

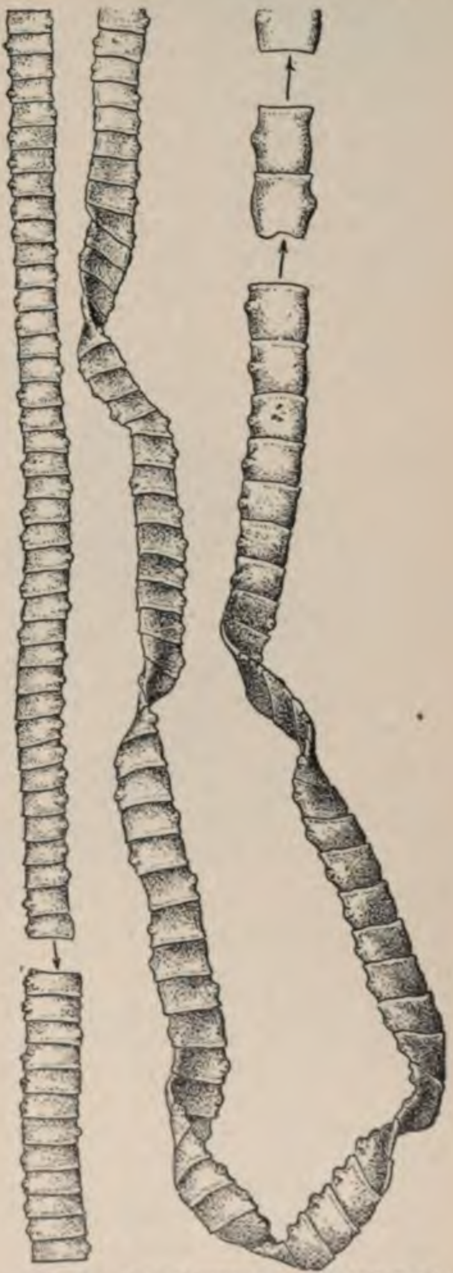


Fig. 151.—Several portions of adult pork-measle tape-worm—Tænia solium (natural size) (Stiles, Bullet. 15, Division of Animal Industries, U. S. Dept. of Agriculture).

of the civilized world, and is especially common in North America. The length of this parasite may at times be practically incredible. In my own experience I have seen a parasite 36½ feet long, which was obtained from an inmate of the Philadelphia Hospital. There are many records of cases in which much longer specimens of the Tænia saginata have been recovered from man.

Head.—The head of the beef tape-worm (Fig. 152) is of a dark-blue color and is said to be much larger than is the head of the Tænia solium. It is best studied under a low-power objective (two-thirds). It has four depressed sucking-cups, but, in striking contrast to the head of the Tænia solium, it has no hooklets; a tapering neck displays a faintly granular surface; slight transverse striations extend from the pigmented portion of the head (Fig. 152).

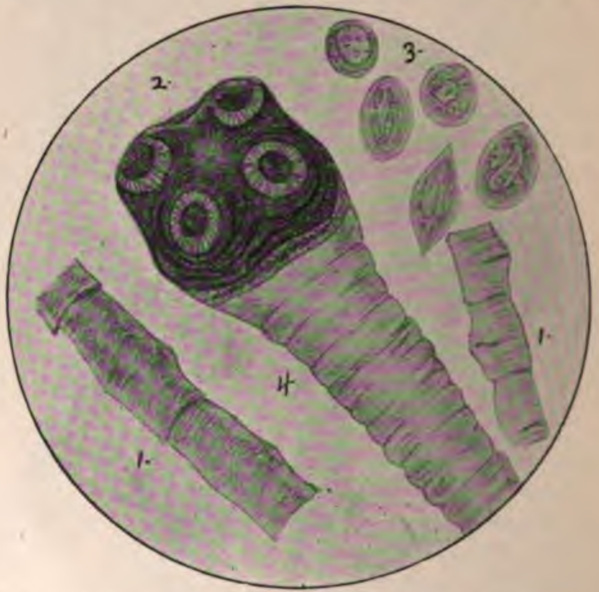


Fig. 152.—Tænia mediocanellata: 1, Large and small segments (natural size); 2, head (obj. B. and L. two-thirds); 3, ova (obj. B. and L. one-sixth); 4, neck.

Proglottides.—The mature proglottides of the beef tape-worm are much broader and thicker than are those of Tænia solium. Segments may escape from the rectum at any time, because they possess a limited power of independent movement. I have placed fresh mature segments of the beef tape-worm and of the dog tape-worm (Tænia marginata) under a two-thirds objective and watched their movements carefully. While such a study is most interesting, it can scarcely be recommended as practical. The individual segment of the beef tape-worm has a central canal (uterus) from each side of which a great number of dichotomously dividing branches are given off (Fig. 153). This is the distinguishing feature between the proglottides of the beef tape-worm and of the pork tape-worm.

Ova.—The ova of the Tænia mediocanellata (Fig. 154) resemble those of the Tænia solium closely. The slight difference in size is not sufficient for diagnosis unless one resorts to measurements. These ova also escape with the feces.

The ova of the tape-worm are best studied by placing a ripe segment upon a slide and tearing a small piece from its center. Place this piece upon a second slide, and apply gentle pressure or tease with a needle to liberate the ova. Discard the tissue

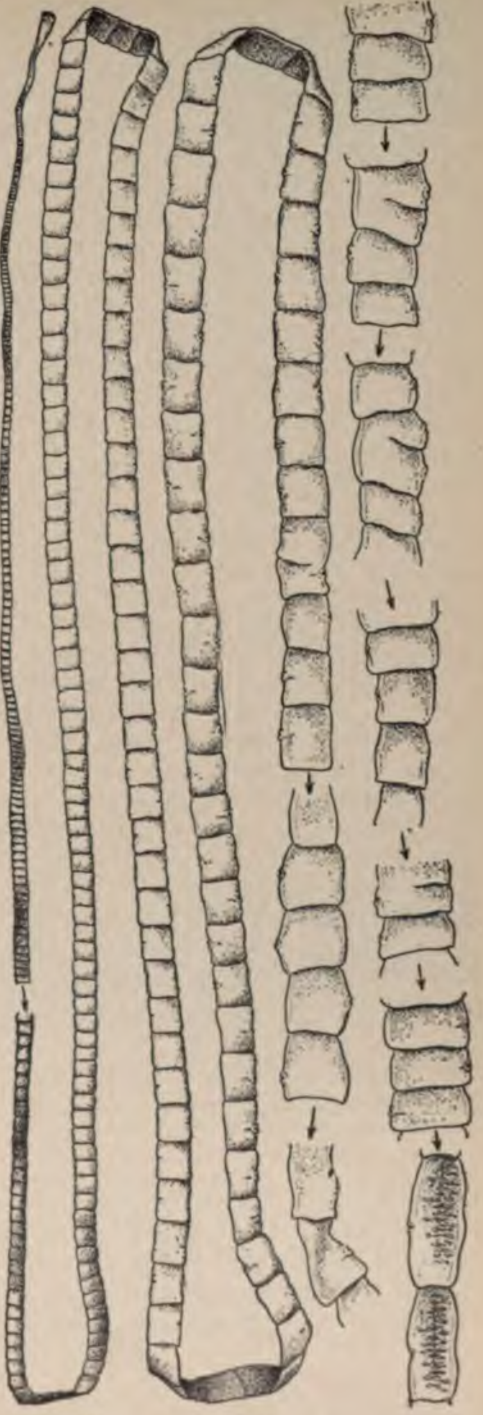


Fig. 153.

Fig. 153.—Portions of adult beef-measle tape-worm (Tænia saginata or Tænia mediocanellata) from man, showing head and gradual increase in size of segments; natural size (Stiles, Bullet. 15, Bureau of Animal Industries, U. S. Dept. of Agriculture).

of the segment, add a drop of water to the liberated ova, and apply a cover-glass (Fig. 154). Specimen smears of the ova of the tape-worm prepared in this manner and fixed by heat (see *Blood*, page 74) stain well with anilin dyes. I have obtained most satisfactory results by staining such specimens for ten minutes with Delafield's hematoxylin.

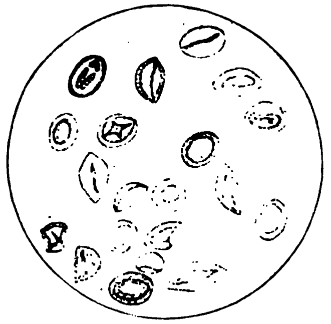


Fig. 154.—Tænia mediocanellata. Ova from the segment (obj. Spencer one-sixth).

Dibothriocephalus Latus.—The Dibothriocephalus latus (Tænia lata of Linné) or fish tape-worm is a form of worm common in all countries bordering upon the Baltic Sea; it is also common in the vicinity of Lake Geneva and in Holland. This parasite, as a rule, appears to infect persons living in damp and marshy districts. Multiple infection may occur, as has been shown by my esteemed friend and teacher, the late Dr. Frederic A. Packard, who reported such a case from the Pennsylvania Hospital. Dr. Robert N. Willson* reports the case of a female infected with at least two bothriocephali. Willson is of the opinion that there were three parasites present, but he was able to find only two heads of the Dibothriocephalus latus. The accompanying illustration (Fig. 155) from Bremser was modified by Willson to represent the parasites recovered by him and to show twin segments, *A* and *B*.

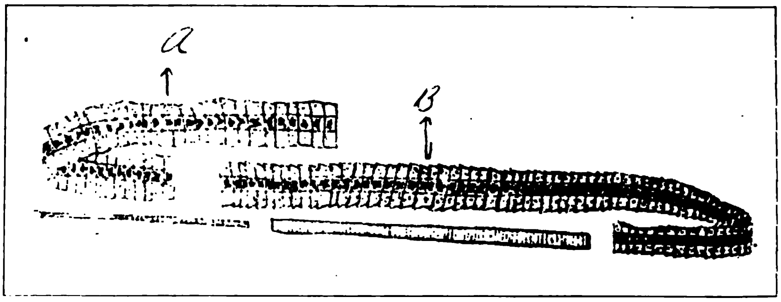


Fig. 155.—Dibothriocephalus latus (fish tape-worm): *A* and *B*, Twin segments (R. N. Willson).

The parasite varies greatly in length, the observations of many writers having established from 2 to 5 meters (6 to 30 feet) as the extremes.

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* "Amer. Jour. Med. Sci.," Aug., 1902.

Head.—The head of the Dibothriocephalus latus is 2 or 3 mm. long by about 1 mm. broad. It is perfectly ovoid (Fig. 150, 4 and 5) in contour, and closely resembles the expanded portion and handle of a spoon. Detection of the head of the dibothriocephalus in the feces is accomplished in the manner described on page 397.

To the naked eye the head of this worm corresponds in size to that of a small pin. It may be of light-gray or pearl-white color; it is sometimes colorless. In striking contrast to both the beef and the pork tape-worm the dibothriocephalus (fish tape-worm) possesses neither hooklets nor suckers. The hooklets, which were present in the early stages of development, are lost before the parasite is matured. There is a narrow groove on each side of the head, which possibly serves for attachment of the parasite to the intestinal wall. "Cutaneous glands are to be found in the head, but are absent in the body." Muscle-fibers and two distinct nerve-cords have also been noted, the latter apearing like two roundish or kidney-shaped spots of granular appearance which gradually approach one another toward the anterior portion of the head and are finally united in a loop by a transverse connection (Leuckart). There is no distinct neck interposed between the head and the small (first) segments.

Proglottides.—The mature proglottides do not escape from the rectum singly, as is observed in the case of the beef tape-worm; but these segments are, as a rule, passed in large numbers—one foot or more of the parasite passing at a time. The individual segment is very thin at a point near the head, as is shown by the accompanying illustration. They gradually increase in size, the largest segments being those farthest from the head. The small segments appear to be greater in length than in breadth, the medium-sized segments being nearly square. The mature segment varies from 2.5 to 4.5 mm. in length, and from 4 to 14 mm. in width.

At the center of each segment there is a dark or slightly bluish spot, indicating the position of the ovary. When studied under a low-power objective (two-thirds), this area appears as an imperfect roset. According to Willson, these rosets deepen in color in proportion to the number of ova contained. In segments from which many or all of the ova have been discharged the rosets are light in color. Rosets and ripe ova are found in the segments, about 500 mm. (19.5 in.) distant from the head.

Ova.—Fully matured segments show the body of the uterus to be so packed with ova that the center of the segment protrudes slightly.

When there exists infection with Dibothriocephalus latus, the feces will be found to contain the ova of this parasite; and when detected in the stool of man, they are of great diagnostic value. These ova are elliptic or ovoid in contour, and, as a rule, of a muddy-white, brownish-white, or brown color. The ova of the dibothriocephalus vary from 0.06 to 0.07 mm. in length, while the width of the ovum is usually equivalent to about one-half that of its length. At one end of the ovum it is possible to discern, by the aid of a one-sixth or one-eighth lens, a faint hyaline band which outlines an apparent lid (Plate 27). This lid may be either closed or partially opened. In this connection it may be well to refer the reader to a diagram of the ova of the Trichocephalus dispar (Plate 26). Upon two occasions I have been asked to examine feces wherein a diagnosis of infection with the bothriocephalus had been made, and in both cases I found ova of the trichocephalus present only.

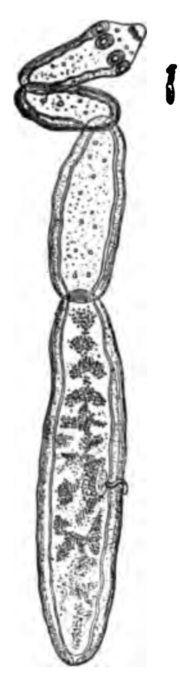

Fig. 156.—Tænia echinococcus, enlarged. Above, at the right, echinococcus of natural size (after Heller).

Tænia Echinococcus.—The Tænia echinococcus, or small dog tape-worm, does not infest the intestine of man, but is commonly to be found in the dog, wolf, and fox. On account of the fact that the larval stage (Echinococcus polymorphus) of this parasite often develops in man, causing the so-called hydatid cyst, it may be of value to present the accompanying illustration (Fig. 156) of the adult worm.

Characteristics.—The Tænia echinococcus is about one-fourth of an inch in length. It is composed of but four segments, including the head. The cephalic extremity, which is prolonged to form a well-marked net, is capped by a pointed rostellum. In the center of the head are well-marked sucking-cups (Fig. 156). The rostellum is surrounded by a double row of hooks, numbering between 30 and 40. The final segment, when sexually mature, is as long as the three anterior segments; it is provided with papillæ at the margin of the proglottis, below the central line. The uterus, which is stuffed with ova, may easily be outlined under a two-thirds objective.

Detection in Man.—The problem of the detection of this parasite in man is radically different from that for the detection of other tape-worms, because man is the intermediary host, and only the head or scolex of the parasite develops. When the scolex develops in the human tissues, it forms a lesion known as a hydatid cyst. Each larval scolex is provided with a crown of hooklets (Figs. 122–124). Free hooklets and shreds of laminated, finely granular, yellowish membrane are usually found in the fluid obtained by puncture of these hydatid cysts. In searching for the products of the Tænia echinococcus a low power of illumination is necessary. These hooklets and scolices are detected by placing a drop of the suspected material upon the center of a slide and studying it under a two-thirds objective. It is more satisfactory to add a cover-glass to such specimens, and to bring the individual scolex into focus under a one-sixth or one-eighth objective, when it will be found to display the characteristics set forth in the accompanying illustrations. Products of the Tænia echinococcus are well preserved when mounted in cast medium, Farrant's medium, and in glycerin-jelly.

Clinical Significance.—"This species, in its larval conditions, is probably more injurious to the human race than all the other species of entozoa put together; or, to say the least, it is more frequently the immediate cause of death than any other internal parasite" (Cobbold).

The finding of hooklets, scolices, or membrane of the Tænia echinococcus is positive evidence of infection with this parasite, and that the larval parasite (scolex) has developed within the human organism. It is comparatively common to find evidence of echino-

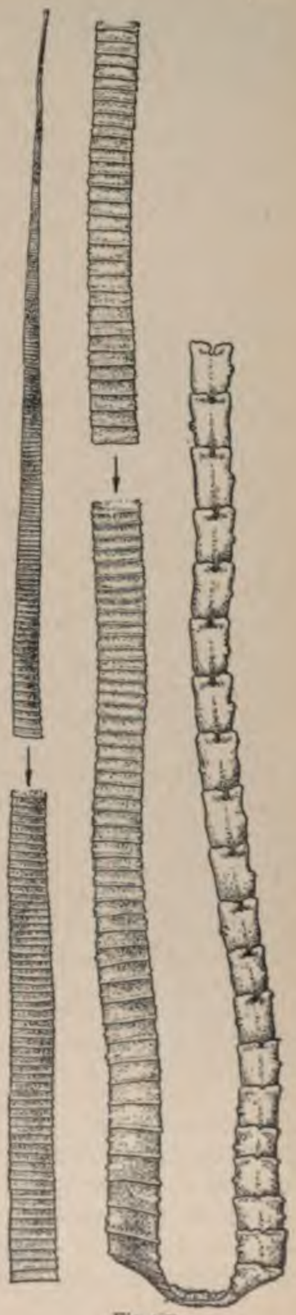

Fig. 157.

Fig. 157.—The marginate tape-worm—Tænia marginata—common in the dog (natural size) (after Stiles).

coccus infection in the contents of cysts and abscesses of the liver, lung, and kidney. The scolices develop in the brain and meninges, but they are less common. The products of a hydatid cyst should always be sought for in the sputum, and I have found them twice in the urine (page 292).

Tænia Marginata.—This parasite (Fig. 157), a species common to the dog, has been known to infest man in but few instances.

Hymenolepis Nana.—The Hymenolepis nana (Tænia nana), or dwarf tape-worm, is occasionally encountered in the intestinal canal of man, but is far more common in the intestine of the lower animals. This parasite is rare in America, but common in Italy, Egypt, and along the Mediterranean. Young individuals are oftenest infected, and display the general symptoms caused by other tape-worms. In length the Hymenolepis nana will be found to vary between two and four inches (Fig. 158). Its characteristic outline, except its head, is practically that of the larger tape-worms.

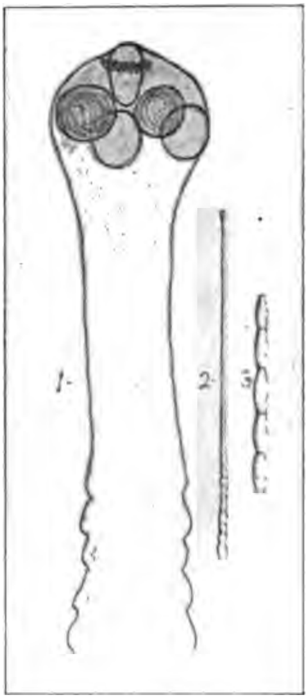

Fig. 158.—Hymenolepis nana from intestine of a cat (personal observation): 1, Head and neck (obj. B. and L. two-thirds); 2, head and neck (natural size); 3, largest segments (natural size).

Head.—The head of the Hymenolepis nana differs radically from the heads of other tape-worms herein described, since it is more or less pear-shaped and displays four suckers and a club-shaped rostellum (Fig. 158). It also contains from 24 to 30 hooklets, which are arranged in a single row to form a crown at the anterior portion of the head. I recovered at postmortem 96 worms (Hymenolepis nana) from the intestinal canal of a cat that had been used for experimental feeding. All these worms were alive when removed from the cat, and while yet warm, many of them were studied microscopically. About half the number of parasites showed the club-shaped rostellum projected from the head, yet the accompanying illustration, which was made six hours later, does not show this condition.

Proglottides and Ova.—The proglottides of the Hymenolepis nana are described by the accompanying illustration. The ova are numerous and are slightly opalescent, oval bodies enveloped in a rather distinct membrane. These ova measure from 0.039 to 0.060 mm. in diameter. In my series of studies I was unable to distinguish satisfactorily an embryo within this capsule, although a six-hooked embryo is said to occupy each mature ovum.

Infection with the Hymenolepis nana is supposed to occur through the eating of certain snails in which the cysticercus form of the parasite develops.

Tænia Cucumerina.—Under this caption has been described a dwarf tape-worm which resembles in many respects the Hymenolepis nana. This parasite is said to infect children and the lower animals, especially the cat and the dog. It resembles the Hymenolepis nana in that it displays a well-marked rostellum around which the hooklets are arranged in rows. In two specimens of tape-worm determined to be the Tænia cucumerina and recovered by me from the intestine of a cat it was difficult to detect any marked difference between these parasites and the specimens of the Hymenolepis nana. The cysticercus form of the Tænia cucumerina is said to develop in flies.

Tænia Flavopunctata (**Hymenolepis diminuta**).—This is a rare intestinal parasite of man. It was first described by the late Dr. Leidy as occurring in the human feces. In 1900 an elaborate study of this parasite, with the report of a ninth case of infection in man, appeared from the pen of the late Dr. Frederic A. Packard.*

In length this parasite will be found to vary between 25 and 60 mm. The head is provided with two rather well-marked sucking-cups. The ova are quite similar to those of the Tænia solium. The cysticercus form of the parasite develops in cocoons and certain caterpillars.

Dipylidium Caninum.—The Dipylidium caninum, or double-pored dog tape-worm, belongs to a family of intestinal parasites rarely encountered in man, but common to the cat and dog. Dr. Charles Wardell Stiles, in addition to reporting an example of infection in a child, has described this parasite in detail.† The parasite is best described by the accompanying illustrations, taken from Stiles (Figs. 159, 160). Its segments, which are elliptic, elongated, tape-like bodies, need not in any way be confused with those of other intestinal parasites (Fig. 159). The adult parasite commonly infests the lower animals, as above mentioned, and the larval stage of the worm develops in lice and in fleas (Fig. 161). Stiles states that the Dipylidium caninum is

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* "Jour. Amer. Med. Ass.," Dec. 15, 1900.

† "Amer. Med.," Jan. 10, 1903, p. 65.

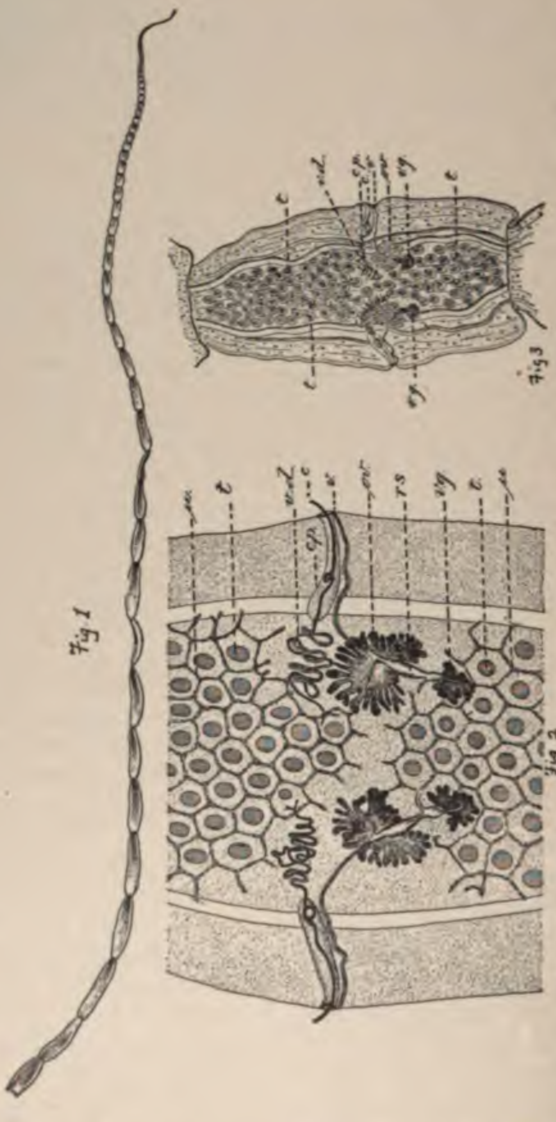

Fig. 159.—Dipylidium caninum: Fig. 1, Adult strobila (natural size); Fig. 2, segments (Stiles) (highly magnified); Fig. 3, ground segment (after Diamari).

one of the smaller tape-worms, but should be looked upon as a pathogenic parasite, and that it sometimes burrows into the intestinal mucosa. The head of the parasite shows four sucking-cups and a rostellum which is surrounded by four rows of hooklets.

Ova.—A microscopic examination of the feces does not yield positive information in all cases of infection with the Dipylidium caninum, since ova are not always present, and when present, are of small size and may be but few in number. In this respect infection with the Dipylidium caninum differs radically from infection with the Tænia solium, Tænia saginata, and the Dibothriocephalus latus.

The individual ovum of Dipylidium caninum is widely different from that of any other form of tenia known to infest man. "In the genus tenia we find a thick, striated inner shell (embryophore), while in dipylidium the inner shell is thin" (Stiles). A six-hooked embryo is to be observed in each egg (Fig. 161).

Tænia Madagascariensis (*Grenet*).—A form of tape-worm which has been found to infest man residing on the eastern coast of Africa. This parasite may attain a considerable length and its segments reach a maximum number of 600. The distinctive features of this parasite are trapezoid segments; a double row of hooklets surrounding its rostellum, and its well-defined sucking-cups. Its intermediary host is unknown.

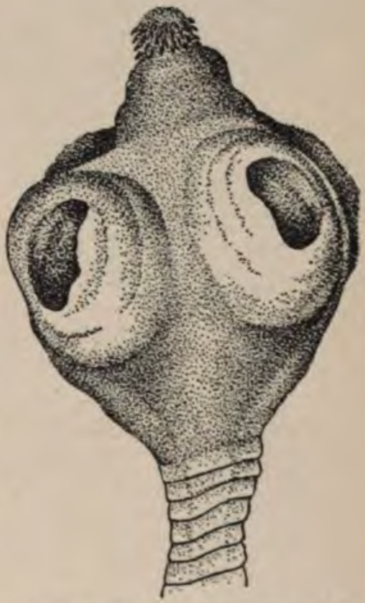

Fig. 160.—Head of Dipylidium caninum, showing four rows of rose-thorn hooks on the rostellum and four unarmed suckers (Stiles).

Tænia Africana.—A parasite detected in the human intestine of man. The host had resided in the vicinity of Nyasa Lake. Linstow* reports the parasite as being devoid of hooklets. It may vary greatly in length, and its segments are broader than they are long, and are usually stuffed with ova. The branches of the uterus are not divided dichotomously.

A Questionable Intestinal Parasite.—Numbers of the following described parasites have been found in the feces of several cases of epilepsy. I am not aware that this parasite has been recovered from the human feces except in the United States along the tributaries of the Mississippi River. This parasite has been studied by G. H. French.*

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* "Centralbl. f. Bakt. u. Parasit.," 1900, vol. xxviii, p. 485.

The adult worm is translucent, measures about ½ inch in length, and is shown in the accompanying illustration (Fig. 162). Its anterior end bears two black hooks which are slightly divergent, and beneath these hooks is a black central line, a suggestion of an enteric canal. The ectoderm is distinctly divided into twelve segments, but no corresponding markings are shown in the endoderm. The incisures, with the exception of the first three, have a band of short spines that project backward. The posterior segment is somewhat villous at the end. A cluster of spines is also seen beneath the points of the hooks. French declines to classify this parasite.

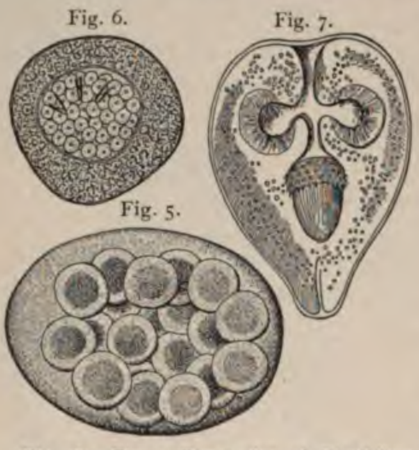

Fig. 161.—Fig. 5, Egg packet of Dipylidium caninum; Fig. 6, egg of same—six-hooked embryo (after Stiles); Fig. 7, Cryptocystis tricodectis, as found in the flea (after Leuckart).

Helophilus.—The helophilus or eristalis (Fig. 163) was recovered by me from the feces of a child which had been in rather ill health for some weeks. During this illness the child had become markedly nervous, and had also experienced slight convulsions. The mother claimed that the child had passed from one to twelve parasites at each bowel movement for a period of several weeks, that the specimen from which the accompanying illustration (Fig. 163) was made was a fair representative of the worms previously passed.

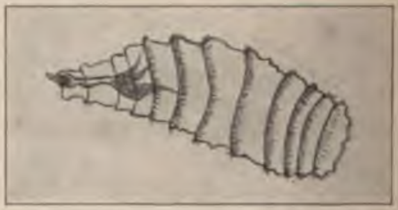

Fig. 162.—Parasite from feces in a case of epilepsy (after G. H. French).

The parasite was determined by Dr. Ch. Wardell Stiles, Bureau of Animal Industry, Washington, D. C., who writes me in part as follows: "The specimen which you sent me for determination . . . is the larval stage of some insect, probably a species belonging either to the genus Eristalis or Helophilus."

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* "Jour. App. Mic.," Rochester, Dec., 1900, p. 1089.

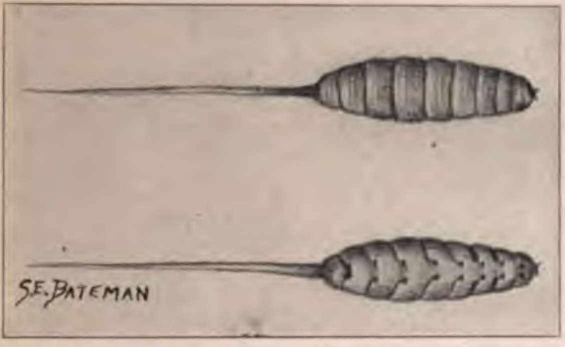

Fig. 163.—Helophilus or eristalis (recovered from feces of a child—an epileptic).

Blue-bottle Fly.—The larvæ of this insect (Fig. 164) are often found in the feces of man residing in tropic districts. This ringed, cylindric parasite is from ½ to 1 inch long, and its body is covered with fine spines or hairs.

Neven-Lemaire* mentions nineteen species of diptera whose larvæ may be found in the intestine or the feces of man.

TREMATODES OR FLUKES.

Fasciola Hepatica.—The common liver fluke is best described by the accompanying illustration (Fig. 165). At its tapering extremity (head) the parasite is provided with a single sucker. A second sucker is at times detectable upon the ventral surface, and situated between these sucking-cups is to be seen the genital opening which leads to the uterus.

The ova of this parasite are ovoid in contour and measure about 0.13 by 0.8 mm. These ova are brown in color, and are provided with an apparent lid at one extremity (Fig. 165). This parasite is not uncommon in sheep, swine, and other animals, as well as in man. In rare instances the ova appear in the feces.

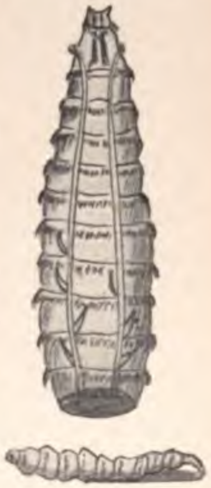

Fig. 164.—Larvæ of the blue-bottle fly (Musca vomitoria) as found in feces (after Leuckart).

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* "Parasitologie Animale."

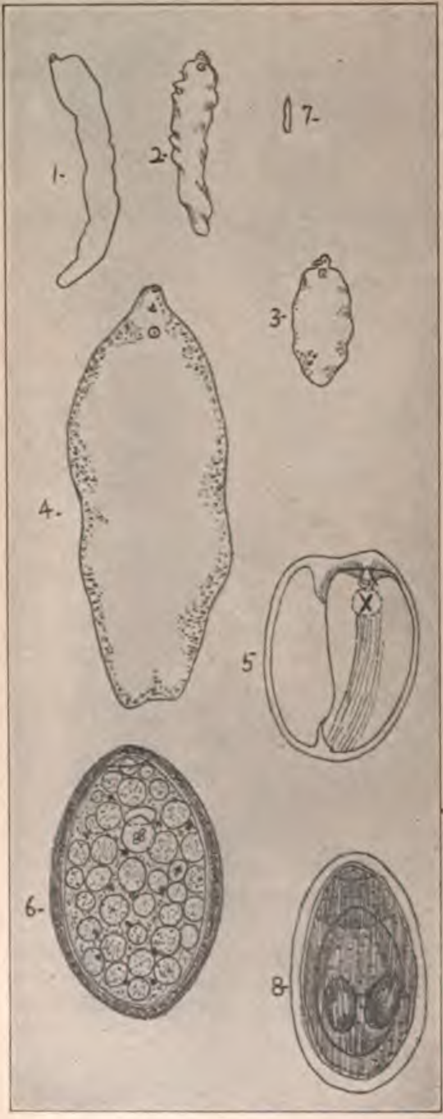


Fig. 165.—1, Narrow liver fluke—Fasciola hepatica angusta (after Stiles). 2, Egyptian liver fluke—Fasciola hepatica ægyptiaca. 3, Common liver fluke—Fasciola hepatica (after Stiles). 4, Large American fluke—Fasciola magna (after Stiles). 5, Ciliated embryo (miracidium) of large American fluke within egg-shell (after Stiles). 6, Egg of large American fluke showing germ-cells surrounded by large number of vitelline cells; egg-shell provided with a cap. 7, Lancet fluke—Dicrocœlium lanceatum (after Stiles). 8, Egg of lancet fluke with embryo (highly magnified) (after Leuckart).

Dicrocœlium Lanceatum (*Lancet Fluke*).—The Dicrocœlium lanceatum is a parasite which rarely infests man, and, so far as I am aware, has been detected only along the Baltic Sea. It will suffice to say that this parasite closely resembles the Fasciola hepatica, except that it is much smaller (Fig. 165). Its ova are also quite small (Fig. 165), and are said to contain well-developed embryos.

Fasciola Hepatica Angusta (*Narrow Fluke*).—This parasite is probably a variety of the liver fluke common to man (Fig. 165). It has been found in cattle slaughtered at St. Louis. Blanchard regards this form as identical with Fasciola gigantica.

Fasciola Hepatica Ægyptiaca (*Egyptian Fluke*).—The Egyptian liver fluke is also to be considered in a study of the trematodes known to infest the human liver and bile-passages. This parasite has been recovered from the giraffe, and question has arisen as to whether or not the Egyptian fluke is identical with the narrow fluke, Fasciola hepatica angusta, previously described. Cattle and man are also probable hosts for this parasite.

Fasciola Magna (*Large American Fluke*).—According to Stiles, the large American fluke (Fasciola magna, Fig. 165) is apparently more common in the United States than is the common liver fluke (Fasciola hepatica). According to Dinwiddie, in certain sections, particularly in Arkansas, a large percentage of the cattle are infected with this organism. Bassi noted that this parasite caused a fatal disease in deer. I recovered three specimens of this parasite from the liver of a Virginia deer.

Distoma Sinense (Opisthorcis sinense).—MacConnell was the first one to describe the Distoma sinense as infecting man in the East Indies. At present this parasite is rather generally known throughout Japan, Corea, Formosa, and China. The Distoma sinense varies in length from 20 to 22 mm., and is leaf-shaped. This parasite is of a reddish shade and, at times, fairly translucent.

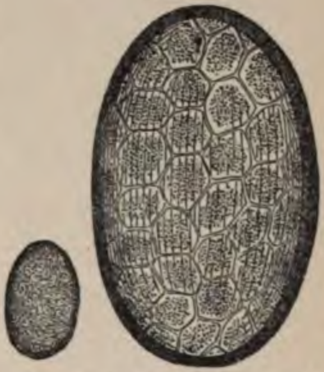


Fig. 166.—Eggs of Distoma hepaticum and Distoma lanceolatum, moderately magnified (Heller).

The ova vary in length from 28 to 30 μ, and are from 15 to 17 μ at their greatest breadth (Fig. 167). In each ripe ovum it is possible to detect a ciliated embryo.

Clinical Significance.—According to Manson, the Distoma sinense invades the bile-ducts and the gall-bladder, causing a thickening of the biliary canals, which become greatly expanded in places to form diverticula. In these cavities numbers of parasites are to be found. Each diverticulum communicates with a bile-duct. The ova (Fig. 167) and rarely the adult parasites may escape through the biliary canals into the intestine and appear in the feces.

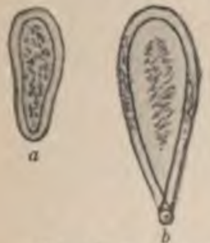


Fig. 167.—*a*, Ovum of Distoma sinense; *b*, parasite, natural size (P. Manson).

Baels states that in the low, marshy districts of Japan, where the hygienic surroundings are especially bad, he found 20 per cent. of the population to be infected with this parasite. Diarrhea and recurrent attacks of jaundice are prominent features of the disease.

ROUND-WORMS.

Ascaris Lumbricoides.—The Ascaris lumbricoides is readily detected, when present in the feces, from the fact that it resembles closely the ordinary earth-worm (Plate 26). Ascaris lumbricoides infests the small intestine, but under rare conditions it may enter the common bile-ducts, the gall-bladder, stomach, mouth, nasal cavity, and appendix. In three specimens of vomitus collected from as many patients I found this adult parasite present. Specimens of Ascaris lumbricoides will be found to vary greatly in size, the female being by far the larger and displaying certain characteristics shown in plate 26. This parasite is found most often in children, and occasionally the worm may escape from the rectum independent of the bowel movement. Cases are recorded where the parasite has crawled from the mouth and from the nose of a child during sleep.

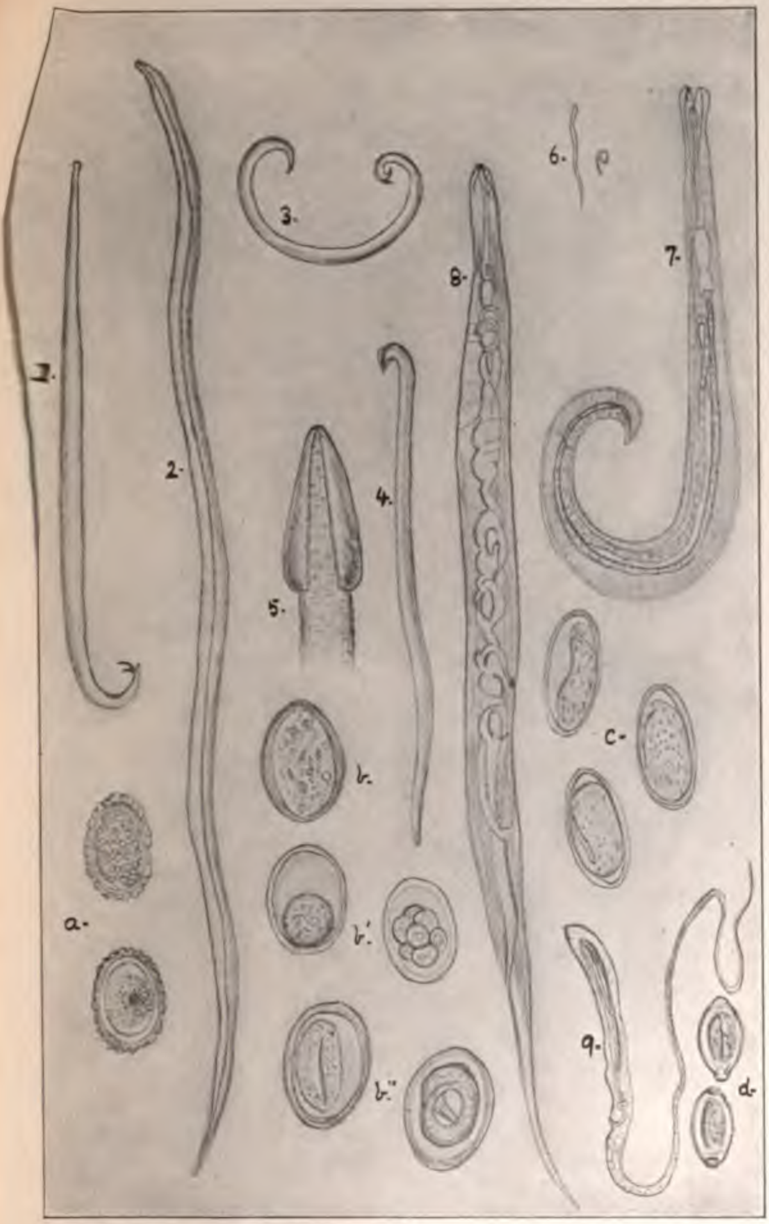
Ova.—Ova of the Ascaris lumbricoides are to be obtained from the material collected by the introduction of a catheter into the rectum (see *Ameba*, page 388). The individual ovum is of a yellowish-brown color, nearly round, and varies from 0.06 to 0.07 mm. in diameter; and when studied under a one-sixth or a one-eighth objective, will be found to display a more or less well-marked double outline (Plate 26). In certain instances the ovum will be found to contain a well-defined embryo (Plate 27). The shell of these ova is hard and very resistant.

Ascaris canis.—The Ascaris canis (A. mystax) resembles in many respects the Ascaris lumbricoides, but is much smaller (Plate 26). When placed under a low-power objective (two-thirds), the head of the Ascaris canis is found to present a fan-like projection from each side, a feature which distinguishes the adult Ascaris canis from the smaller Ascaris lumbricoides. The adult female Ascaris canis will be found to vary from 100 to 120 mm. in length. The male worm is about one-half this size.

Ova.—Ova of the Ascaris canis resemble to some extent those of Ascaris lumbricoides, and are best described by the accompanying illustrations. It is not rare to obtain the Ascaris canis from the intestine of the cat at postmortem.

Oxyuris Vermicularis.—The Oxyuris vermicularis infests the lower portion of the intestine and the rectum, and occasionally migrates to the vagina and bladder. Children are more often affected than are adults. I have records of fourteen cases of infection occurring in persons beyond the age of forty. It is usually possible to collect the worms from the anus of the

PLATE 26.



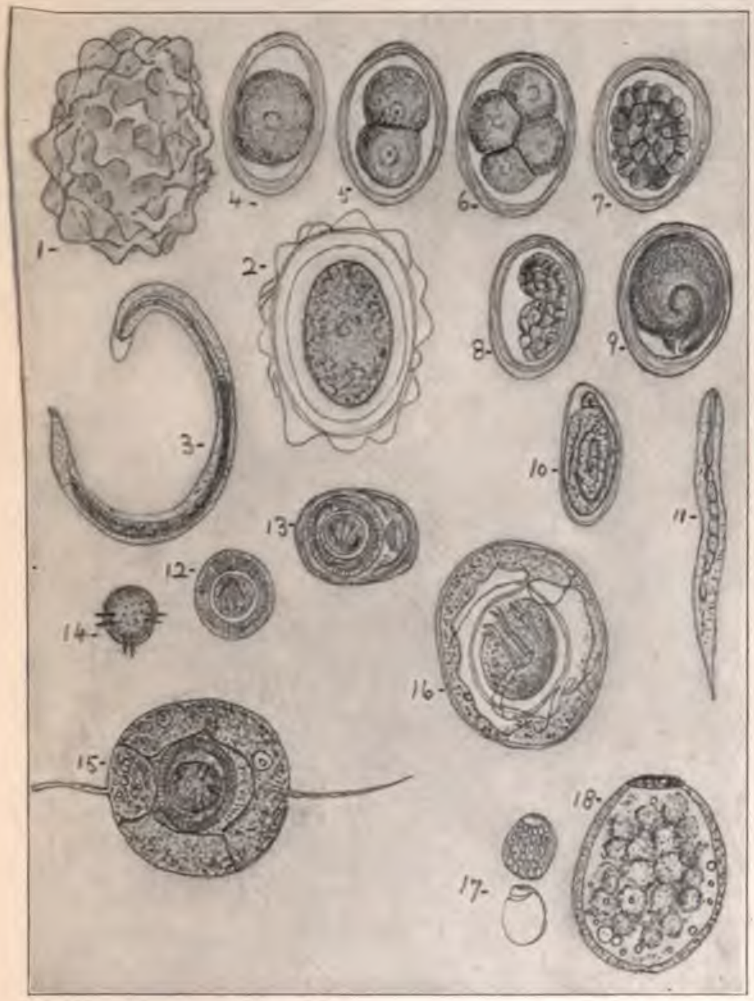
1, 2, and *a*. Ascaris lumbricoides: 1, Male; 2, female; *a*, ova.

3, 4, 5, *b*, *b′*, *b″*. Ascaris canis: 3, Male; 4, female; 5, head of female (magnified); *b*, ovum; *b′*, ova, showing segmentation; *b″*, ova showing embryo (Kobbold).

6, 7, 8, and *c*. Oxyuris vermicularis: 6, Male and female (natural size); 7, male; 8, female (magnified); *c*, ova (personal observations) (obj. B. and L. one-sixth).

9 and *d*. Trichocephalus dispar: 9, Female (magnified); *d*, ova (obj. Queen one-sixth).

PLATE 27.



1. Egg of common Ascaris lumbricoides (superficial focus; Stiles). 2. Same, median focus greatly enlarged (Stiles). 3. Free embryo, common Ascaris lumbricoides casting its skin (Leuckart). 4–9. Embryology of Ascaris lumbricoides, after egg is deposited in feces (Leuckart). 10. Ovum of Oxyuris vermicularis, showing embryo (Leuckart). 11. Full-grown free embryo of oxyuris (Leuckart). 12. Egg of pork tape-worm (Tænia solium) without primitive vitelline membrane, showing striated embryophore (Leuckart). 12. Egg of same, with primitive vitelline membrane. 14, 15. Egg of beef tape-worm (Leuckart). 16. Egg of Hymenolepis nana (Ransom). 17. Egg of the Dibothriocephalus latus, showing lid. 18. Egg of same, highly magnified.

patient, and when thus collected and placed in warm water, they will live for a number of days.

The female worm will be found to measure between 5 and 10 mm. in length, and the male is scarcely more than half this size. From the sides of the head of the oxyuris there is a peculiar projection (Plate 26). The extremities in the male and female parasite are decidedly different (Plate 26). It is claimed that the female parasite locates in the cecum prior to impregnation; but my own observations do not coincide with this statement. In every case of pruritus ani observed resulting from oxyuris infection I have found both male and female parasites present; and many of the female parasites were not fully matured and their uteri not filled with ova.

Ova.—A most interesting study is to place the female worm upon a previously warmed slide; add sufficient water partially to cover her, and then examine under a low-power objective (two-thirds). The rhythmic contractions of the uterus are in this way seen, and with each contraction one or more ova escape from the vagina. At times many ova are expelled at a single contraction of the uterus, and these are often entangled in a net-work which resembles a fish's spawn. To obtain ova of the oxyurides for laboratory study place several adult female worms in a small bottle; add one or two ounces of water, cork with cotton, and place at incubating temperature for from twelve to twenty-four hours. Lift the sediment from the bottom of this bottle into a pipet and place a drop of it upon the center of a slide; add a cover-glass and examine under a one-sixth objective, when numbers of ova will be seen (Plates 26, 27).

The ovum of the oxyuris is about 0.05 to 0.03 mm. The operculum, or envelop of this ovum (Plate 27), is thin and asymmetric, one side being nearly straight. It is possible to detect in some of the ova a rather clearly formed embryo.

Ova of the oxyuris are common in the feces of children infected with this parasite, and I have repeatedly found them in the vaginal secretion and in the urine of such children.

Uncinaria (Ankylostoma).—This parasite invades the intestine of man, and as a result of such infection a group of symptoms are produced which are spoken of collectively as uncinariasis, ankylostomiasis, or hook-worm disease. The total length of the female worm will vary between 10 and 18 mm., while the male worm is but slightly more than one-half this length. The tapering condition of the neck and head, which is slightly turned, is described by figure 168, as is also the tail of the female worm, which tapers gradually, terminating at a slightly rounded point.

The naked-eye appearance of the Uncinaria americana and the Uncinaria duodenale (old-world hook-worm) is quite like that of the oxyuris, but when studied under a two-thirds objective, both the head and the tail of the uncinaria will be found to differ radically from those of the oxyuris. These features are made plain by the accompanying illustrations. The tail of the male parasite displays several distinct cilia, or hairs (Fig. 168), and a three-lobed bursa which serve to differentiate the uncinaria from other intestinal parasites. The mouth of the uncinaria is also quite characteristic (Fig. 168). The uterus of the female worm is readily outlined with a two-thirds objective, and when

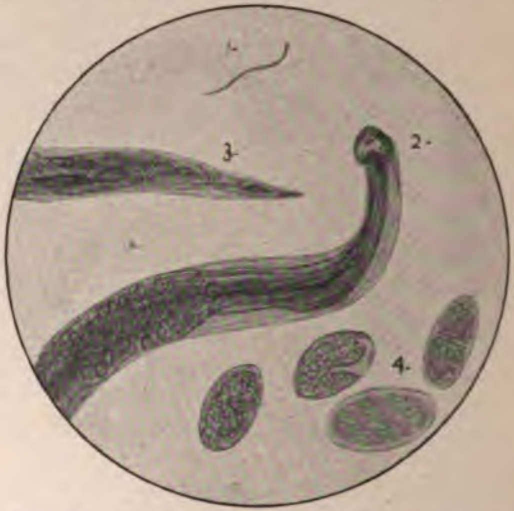


Fig. 168.—Uncinaria americana: 1, Female, natural size; 2, head; 3, tail; 4, ova (B. and L. obj., eye-piece 2).

studied under a slightly higher power objective (one-sixth), is seen to be filled with ova (Fig. 168).

Detection in the Feces.—Uncinariasis may exist for a long time and yet there be no parasites in the feces. In such instances it becomes necessary to administer some drug for the purpose of expelling these parasites from the intestinal tract. Before the administration of an anthelmintic it is well to give the patient sufficient citrate of magnesia to empty the bowel thoroughly. The stomach must also be empty. The drug which serves best for the expulsion of the uncinaria is thymol, which can be administered in doses of 2 gm. (31 gr.) at 8 A. M. and this dose repeated at 10 A. M.

Caution.—It is necessary that a careful watch be kept over the patient after the administration of the first dose of thymol, since this drug often acts as a decided cardiac depressant. Whenever its depressing effects are evident, the heart should be stimulated with strychnin or alcohol (alcohol is said to favor the absorption of thymol by the system). The administration of the drug is not to be continued in such instances until the heart has first been strengthened by proper cardiac stimulants.

Two hours later—12 o'clock M.—follow the last dose of thymol with a liberal dose of magnesium citrate or of castor oil, and collect all the feces. Worms are found in the stools in from eight to twelve hours after the administration of thymol. Add water to the feces, mix thoroughly, and strain through gauze, as described under the *Detection of the Tape-worm's Head*, page 397. In this manner it is very easy to detect the worms upon the gauze, from which they should be removed by the aid of a small needle and placed in water. These worms are well preserved in 70 per cent. alcohol; and when dehydrated, may be mounted in Canada balsam. The worms are well preserved after treatment with 50 per cent. alcohol when mounted in cast medium.

Ova of the Uncinaria duodenale are to be found in the mucus obtained by introducing a catheter into the rectum, and are also to be recovered from the feces. The number of ova present in the feces is in direct proportion to the number of parasites in the intestine, yet Ashford states that the feces may contain great numbers of ova when there are but few female worms in the intestine. Ova, when present in the feces, are detected by placing a small portion of the dejecta in a glass and adding to it a rather liberal quantity of water, mixing thoroughly, and centrifugalizing a quantity of this mixture. Sediment collected in this manner and studied under a two-thirds objective will be found to contain ova. Washing of the feces may be of service (page 387).

Ova.—The ovum of the uncinaria (Fig. 168) measures between 0.05 and 0.06 mm. in length by 0.03 to 0.04 mm. in breadth. It is common to see ova rather dark and slightly granular in appearance while others are faintly opalescent. A third variety of ova may be almost hyaline, except for a small portion extending along one side of the capsule, where a faintly granular body is seen—an apparent embryo. Ova showing segmentation (Fig. 171) are extremely common. When the feces is allowed to stand at incubating temperature, the embryos are seen to develop rapidly. and some will have escaped from their ova in from twenty-four to forty-eight hours. The finding of shells of ova showing a slight line or opening is at such times very common (Fig. 171).

The embryos are said to be found in the feces, but personally I have never been able to detect these embryonic worms in such feces.

In mounting ova of the Uncinaria duodenale and americana the feces should be diluted liberally with water and the process of centrifugalization be repeated three or more times, transferring the sediment to a clean tube after each centrifugalization. Ova when washed in this manner should be placed upon the center of a slide and permitted to dry in the air. They may now be mounted in Canada balsam, cast medium, or glycerin-jelly. I have in my possession several slides thus mounted in 1896 and at present these ova show well their characteristic features. The ringing of the specimen with microscopic asphaltum is not an absolutely necessary step in this technic, yet it is my custom to ring all specimens (page 28).

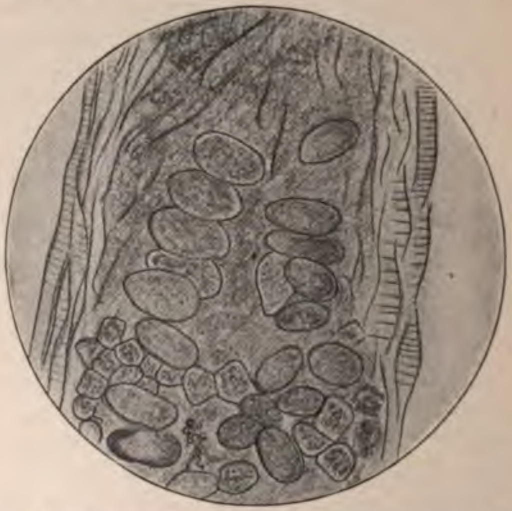


Fig. 169.—Section of adult Uncinaria ankylostoma, uterus containing ova (obj. Queen one-sixth; eye-piece 2).

Clinical Significance.—The detection of either the adult parasite or of its ova in the feces is positive evidence of infection with uncinaria. B. K. Ashford has shown that a large percentage of cases of anemia studied by him in Porto Rico were caused by infection with the Uncinaria duodenale (see *Blood*, page 143).

Trichocephalus Dispar.—The Trichocephalus hominis is a member of a family of trichotrachelides (Plate 26), and inhabits the cecum in man. This parasite will be found to vary between 40 and 50 mm. in length.

Ova.—The ovum of the trichocephalus is of nearly the same size as the ovum of the uncinaria, and measures about 0.05 mm. in breadth by 0.06 mm. in length. The ovum of the trichocephalus is unlike that of other intestinal parasites, since it displays a decided double outline upon each lateral border (Fig. 171). Another feature which serves to distinguish this ovum is the fact that it displays a hyaline or slightly yellowish lid at each extremity. The central portion of the ovum is coarsely granular, as a rule, and in some of the specimens there is seen evidence of segmentation.

Ova of the Trichocephalus dispar may be mounted in the manner described for mounting the Uncinaria duodenale. It is claimed for the Trichocephalus dispar that it is to be found throughout the civilized world.

Clinical Significance.—In the feces from all cases of infection with the Uncinaria duodenale I have found ova of the trichocephalus. Ashford has informed me that these ova are always present in the feces of persons suffering from uncinariasis, and that the degree of anemia caused by the trichocephalus remains an open question. Upon two occasions I have detected ova of the trichocephalus in the urine. and they are commonly met with in the feces of typhoid fever and of dysentery.

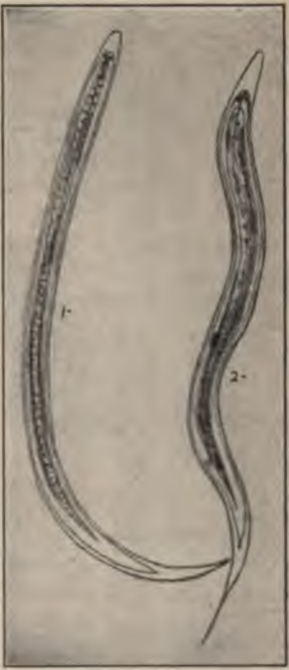


Fig. 170.—Two larvæ of old-world hook-worm, end of second stage (after Perroncito).

Strongyloides Intestinalis.—Specimens of the Strongyloides intestinalis are commonly found in the feces of persons residing in China and in tropic and subtropic districts. Of recent years this parasite has been found in southern United States. Thayer contributed an admirable monograph on this subject.*

The length of the adult female worm is about 1 mm. and its greatest breadth about 0.04 mm. The head tapers slightly, and the tail terminates in a fine point. In the living parasite there are to be seen slight transverse furrows. The mouth displays three distinct lips, and is continuous with a triangular esophagus which becomes constricted. Beyond this, constriction is again expanded to form an ovoid enlargement. The uterus is readily outlined, and is slightly posterior to the center of the body, on the ventral surface of which is a small opening which leads to the uterus. Price * states: "In some instances the young had actually broken the shell and were seen in the uterus, though more often the ova, on deposition, contained well-formed motile embryos."

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* "Jour. of Exp. Med.," Nov. 29, 1901.

The male parasite is slightly smaller than the female (Plate 28). The peri-intestinal cells are accompanied by what appears to be a long gland composed of small globules. This gland, probably a testicle, extends to the base of the tail, where it terminates in two horn-like spicules. These worms are to be found postmortem in the duodenum, jejunum, and occasionally in the ileum. They at times migrate to the stomach, even under apparently normal conditions, and have been found in the vomitus following the administration of an emetic. They are also to be found in the stools.

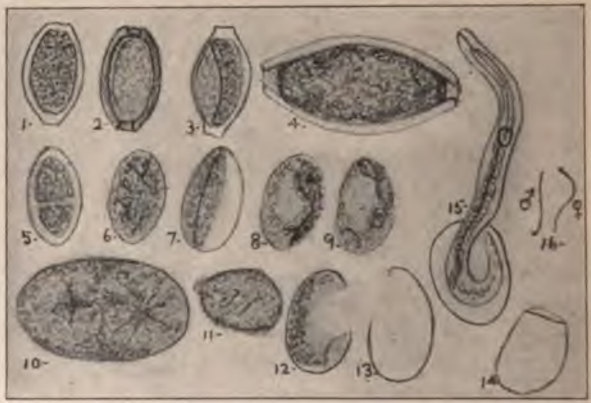

Fig. 171.—1, 2, Ovum of Trichocephalus dispar at different focus; 3, Trichocephalus dispar containing embryo; 4. ovum of Trichocephalus dispar (obj. B. and L. one-twelfth oil-immersion); 5–9, ova of the Uncinaria americana (new-world hook-worm) from feces; 10, No. 6, × 1000; 11, degenerated ovum of Uncinaria duodenale; 12, ovum of old-world hook-worm, showing rupture of shell; 13, empty shell; 14, shrunken empty shells (all from personal observations); 15, escaping embryo (after Perroncito); 16, Uncinaria duodenale, male and female, natural size (after Stiles).

When the stools are kept warm for a variable period, they will be found to contain an embryonic parasite which is much longer, thinner, more homogeneous, and more active than is the ordinary rhabditiform embryo (Fig. 172). This parasite is the strongyloid in its embryonic state. The parthenogenetic female worm (capable of production for several successive generations

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* "Jour. Amer. Med. Ass.," Sept. 12, 1903, p. 651.

without renewed fertilization by the male) also displays a strongyloid type, and possibly develops directly from this strongyloid embryo. The strongyloid embryo, while longer than the rhabditiform, does not attain the size of the parthenogenetic mother-worm. Price has offered the following table of measurements:

	Rhabditiform.	Filariform.	Free Sexualiy.	Parthenogenetic Mother-worm.
Length	0.330 mm.	0.50 mm.	1.00 mm.	2.20 mm.
Breadth	0.022 mm.	0.02 mm	0.04 mm.	0.03 mm.

The ova of the Strongyloides intestinalis resemble in certain respects the ova of the uncinaria, and measure from 60 to 70 μ by 35 to 39 μ.

Strongyloides Subtilis.—Strongyloides subtilis is generally known as an Egyptian and Japanese strongle. In view of our tropic possessions and the frequency with which persons travel in Japan and Egypt, the possibility of this parasite becoming fairly common in Americans is increased. It has not been proved, however, that the Strongyloides subtilis (Plate 28) has not a far wider geographic distribution.

Recognition.—In a general way, according to Stiles, strongylidæ may be determined by the following characteristics: "Body slender; anterior extremity occasionally with alæ. . . . Mouth small, without teeth; lips soft, often indistinct, papillæ very small. . . . Bursa (male) entire or excised ventrally, in some cases bi-, tri-, or multilobed; spicules 2, often with accessory piece." The uterus displays two horns, and the vulva is, as a rule, situated in the caudal half of the body. This parasite will be found to vary between 4 and 7 mm. in length; its oral papillæ are not conspicuous, and its cuticle displays fine transverse striations; longitudinal lines are also present. The lateral longitudinal lines are more distinct than are the central lines. The male parasite is considerably the smaller, and seldom exceeds 5 mm. in length.

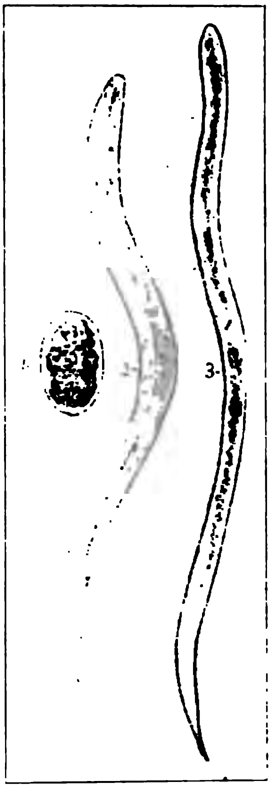


Fig. 172.—1, Egg of the Cochin China diarrhea worm (Strongyloides stercoralis). 2, rhabditiform embryo of same; 3, filariform larva of the same (after Thayer; see also Plate 28).

Ova.—The ova of the parasite are oval in contour, and, according to Stiles, measure about 63 by 40 μ; the shell is thin, and the egg contents are highly granular. Segmentation is not seen in the ova obtained from parasites of a human host. It has not been proved that the detection of the ova in the feces is practical, since it is thought they occur only where a severe grade of infection exists.

Anguillula Intestinalis.—Ova of the Anguillula intestinalis are seldom to be found in the feces, but the embryos of this parasite are commonly found in the dejecta. The ova, when present, are slightly longer than are the ova of the uncinaria, more elliptic, and decidedly more tapering at their poles. The adult worm may appear in the feces.

Trichinella Spiralis.—The Trichinella spiralis is introduced into the system through the ingestion of infected meats in which the embryo worms are encysted. The first symptoms of infection with the trichinæ that concern us from a clinical standpoint are those of gastro-intestinal irritation—nausea, vomiting, and diarrhea. These symptoms do not appear until about one week after the taking of infected meats. I have found these symptoms to appear in a shorter period (three days) where an animal had been fed a large amount of measly pork. It is the rule for these symptoms to be rather mild in man, and for the disease to progress to the stage where the embryos become lodged in the muscle-tissue before it is recognized. Trichinosis is a not uncommon disease throughout Europe and in America.

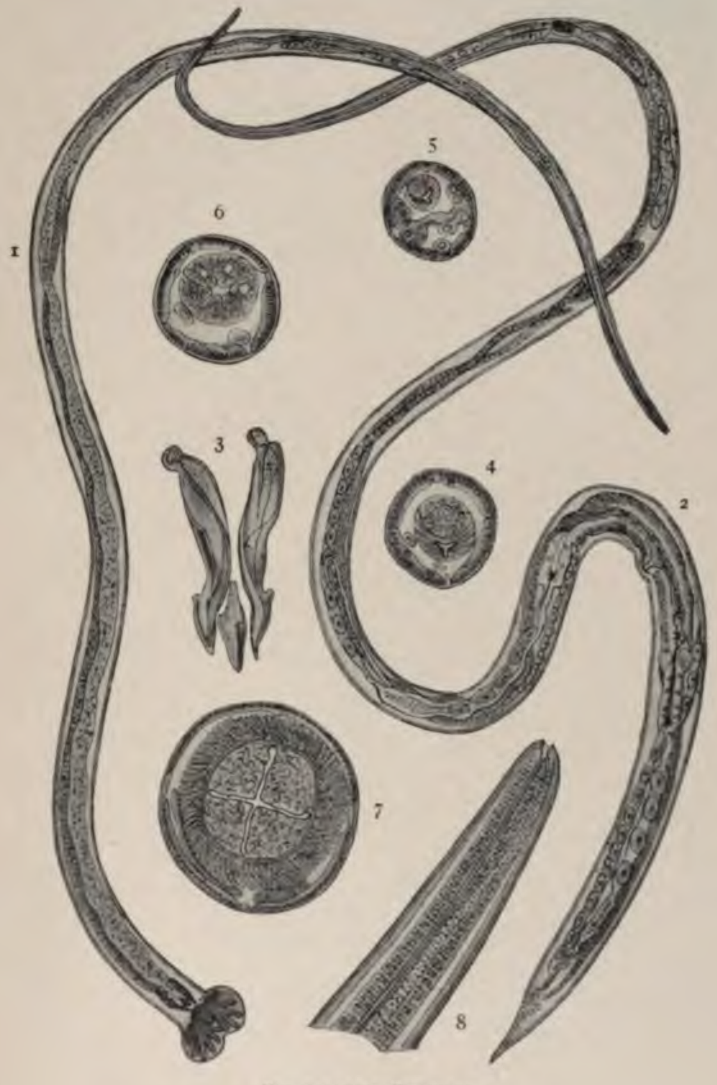
Feces.—The feces, when collected during the paroxysm of gastro-intestinal symptoms, may at times contain the adult trichinæ (Fig. 173); and the detection of this parasite in the feces differs in no way from that outlined for the detection of the Uncinaria duodenale.

Embryos.—Embryos of the Trichinella spiralis are found in the muscles in practically all portions of the body. The accompanying original illustrations (Figs. 174, 175) were sketched from portions of muscles recovered upon the twenty-first day and during the seventh week after the first symptoms of the disease, and figure 176 was obtained one year later.

Detection.—In man a most favorable point of election is the outer head of the gastrocnemius or the tendinous portion of the soleus muscle. An established fact is that the larvæ are far more numerous near the tendinous insertions of the muscles.

1. Cleanse the skin overlying these tendons as though preparing for any surgical operation.
2. Inject into the skin a few drops of a 4 per cent. solution of

PLATE 28.



STRONGYLUS SUBTILIS.

1. Full-grown male.
2. Female.
3. Spicules of male with trowel-shaped accessory spine.
4. Transverse section through middle of esophagus.
5. Anterior, 6, posterior section through vagina.
7. Transverse section through distal part of vagina.
8. Anterior end of female (all greatly enlarged) (Looss).

cocain hydrochlorate. The deeper structures may be injected down to the sheath of the muscle, but in most instances deep injection is not required. In from two to five minutes anesthesia will be found to be complete.

3. Make an incision two or three inches long directly over the tendinous portion of the muscle, dividing the skin and fascia until the sheath of the muscle tendon is exposed. Grasp the sheath of the tendon with a rat-toothed forceps, make a longitudinal incision into the sheath, and keep the sheath separated by grasping each margin of the incised sheath with a forceps. Take up a small portion of the muscle-tissue and dissect it away with the aid of a scissors.

4. Place this small bit of muscle-tissue in a glass containing warm water.

Caution.—Should the muscle be placed in glycerin or in alcohol, the movements of the trichinæ are arrested.

5. Close the wound with two or more sutures, and dress aseptically.

By this method sufficient muscle can be removed for clinical study without danger to the patient. The plunging of a harpoon into the muscle is not advocated.

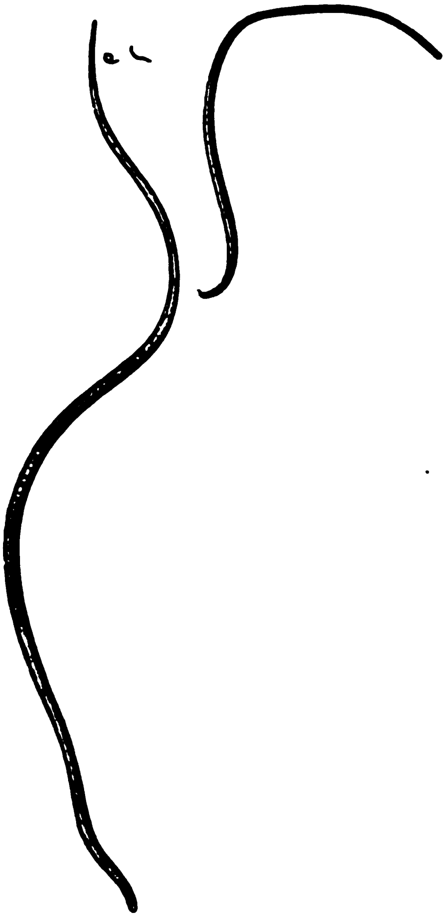


Fig. 173.—Adult intestinal Trichinella (human); male, female, and two embryos, the former natural size, the latter slightly magnified (Birch-Hirschfeld).

Study of the Tissue.—1. Clip a small piece from the mass of tissue, always cutting transversely to the muscle-fibers. Place this clipping on the center of a slide, add to it a few drops of water, and tease the particle of tissue until the fibers are well separated.

2. Without the addition of a cover-glass place the specimen under a two-thirds objective and examine for the larval trichina (Figs. 174, 175). By the addition of a cover-glass the trichina may be studied under a higher power objective—one-sixth or one-eighth.

Trichinæ are best seen with a low illumination, since the parasite is often rendered transparent when the light is too strong. In the process of teasing the muscle-tissue certain parasites are usually freed from the tissue and appear in liquid upon the slide (Fig. 174). During the first two months after infection with Trichinella spiralis the larval parasite becomes actively motile when it is separated from the muscle-tissue. It will uncoil and recoil itself, allowing its more pointed extremity to remain stationary, while the remainder of its body stretches across the field. Whenever this larva comes in contact with any muscle-tissue, it appears to cling tightly to it, at once resumes its usual spiral-like position, and remains quiescent. In the study of a number of parasites no movement was ever detected when the parasite was in contact with the muscle-tissue.

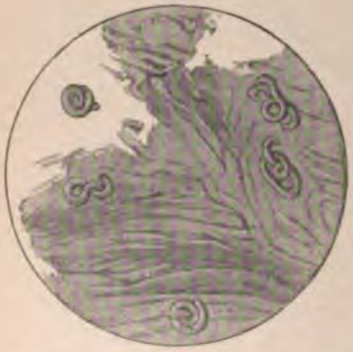


Fig. 174.—Trichinella spiralis (larvæ) from head of right gastrocnemius muscle; seventh week of disease (Queen two-thirds obj.; eye-piece 4).

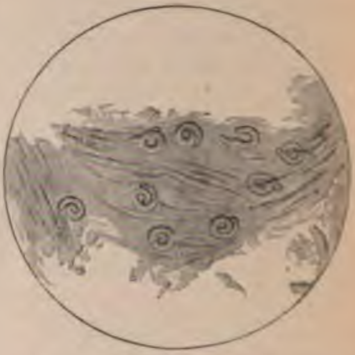


Fig. 175.—Trichinella spiralis (larvæ) from outer head of left gastrocnemius muscle; twenty-first day after symptoms (Queen two-thirds obj.; eye-piece 2).

After a variable period of time these larval parasites become encysted in the muscle-tissue, and at times appear to be within a single muscle-fiber (Fig. 176). Still later the sheath enveloping the larvæ becomes calcified. I found, by freeing the encapsulated trichinæ, that it often possesses slight movement, but the decided movements displayed by the parasite before encapsulation are lacking. I conducted a series of feeding experiments at the Philadelphia Hospital in 1898, and it will suffice to state that cats, rats, and white mice are readily infected with the Trichina spiralis.

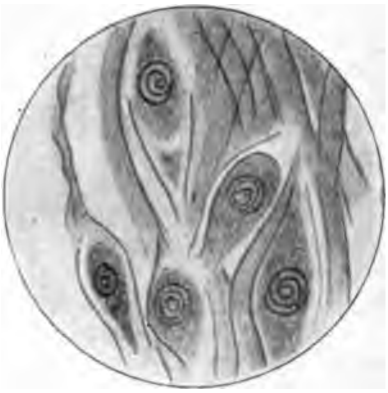


Fig. 176.—Encapsulated trichina from muscle one year after infection.

Permanent Mounts.—When it is desired that permanent mounts be prepared, tease a small portion of a muscle-fiber on the center of a slide, evaporate *nearly* to dryness, and then add to the specimen sufficient cast medium, Farrant's medium, or glycerin-jelly to cover it completely. Then allow a cover-glass to fall gently upon the medium and specimen. Place such mounted specimens in a cool place for twenty-four to forty-eight hours, and ring with some form of microscopic cement. The specimens from which the accompanying illustrations were sketched were mounted in this manner seven years ago, and at present they show equally well all characteristics of the parasite.

CHAPTER V.

THE SPUTUM.

COLLECTION.

Sputum should be collected in a suitable receptacle, for which purpose I find an ordinary vaselin bottle most convenient. This should be placed at the patient's bedside or in his room, so that at the time of the morning exacerbation of coughing he may expectorate directly into the bottle. If possible, the bottle should be filled one-half or more with sputum, when it should be corked tightly and wrapped in a quite heavy paper, upon which the name of the patient is to be clearly written.

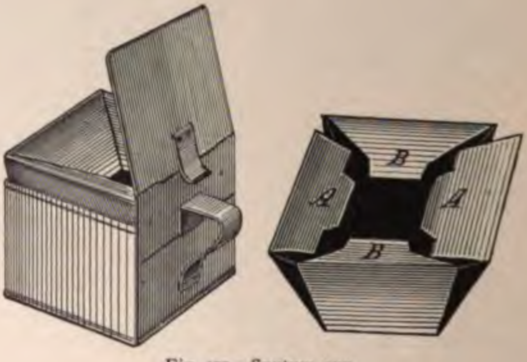


Fig. 177.—Sputum cup.

Caution.—Do not add water to the sputum.

A paper spit-cup has been devised for the collection of sputum at the bedside, and is of special value, since it may be burned after use. I have found an earthen cup, provided with a concave cover in the center of which is a small opening like that in a cuspidor, equally satisfactory. These cups may be disinfected by boiling or by strong bichlorid solution, a feature of greatest importance in any receptacle used to collect sputum.

METHOD OF COLLECTING SPRAY.

This is accomplished by means of a mask (Figs. 178, 179), made from German-silver wire, one piece of which is molded to fit the face, resting on the nose, cheeks, and chin. To obviate any irritation to the patient this portion is covered by a piece of rubber tubing. Suspended from this wire is a second oblong portion provided with two lateral grooves, which serve to accommodate two microscope slides. When the mask is in position, the slides are held directly in front of the mouth and nose, at a point three inches distant from the lips. The mask is held in position by an elastic band which passes above the ears and over the occiput (Fig. 179).

Patients are allowed to wear the mask with the clean slides in position for from one to one and a half hours, during the day, when they are apt to cough least, and are instructed to remove it during a paroxysm of coughing. It is never worn during the morning or evening, the object being not to collect on the slide the spray produced by vigorous coughing, which has already been studied, but to determine whether or not consumptives emit a fine spray, when talking, laughing, clearing of throat, or by their characteristic hacking, that was in any way dangerous to the health of their associates.

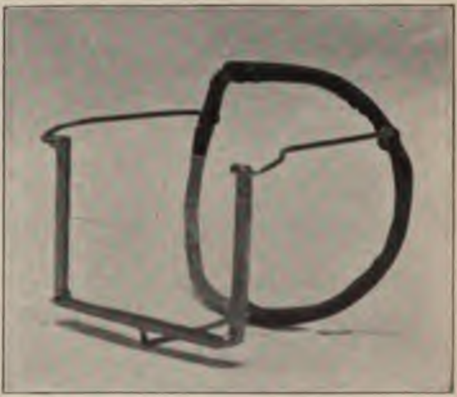


Fig. 178.—Mask with slides in position.

Fifty patients, 34 males and 16 females, all of which presented unquestionable evidence of either pulmonary or laryngeal tuberculosis and in whose sputa tubercle bacilli had been found, were made to wear the mask as above described.

Microscopic Study.—Specimens are fixed and stained by carbolfuchsin and Gabbett's acid-blue solution. Of the specimens collected from 50 patients, those from 49 were found to contain bacteria—the diplococcus and the streptococcus being the most constant, yet bacilli and clusters of cocci were not unusual. A single minute droplet often contained organisms of each class.

Of these 50 specimens, 38 were found to contain tubercle bacilli in variable numbers, 4 to 6 bacilli being the smallest number found in any specimen, and many of the specimens, under a one-twelfth oil-immersion objective, showed fields of bacilli too numerous to be counted.

CHARACTERISTICS OF SPUTUM.

Quantity.—The quantity of sputum ejected during the twenty-four hours will be found to vary greatly, and will bear a direct relation to the nature of the disease in question. The quantity varies from a few cubic centimeters to 500 or even 1000 c.c. a day. Profuse expectoration is to be seen in pulmonary hemorrhage, pulmonary edema, bronchiectasis, tuberculosis, rupture of an abscess into the lung (diaphragmatic, hepatic, pulmonary), and rarely in pleural effusions. The so-called albuminous sputum,

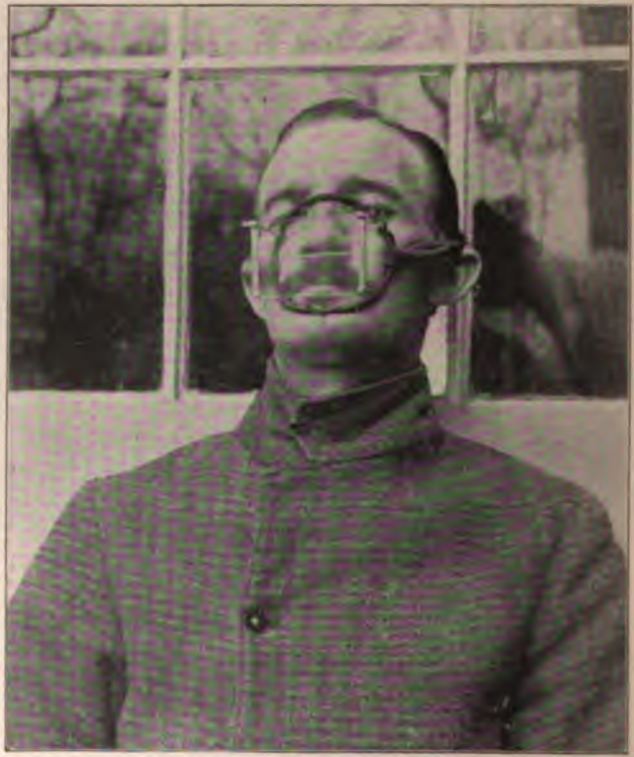


Fig. 179.—Patient wearing mask.

which is observed in gangrene and bronchial blennorrhea, is often profuse. In acute processes the sputum is generally scanty or moderate in amount.

Odor.—The odor of the sputum can be regarded as a characteristic in but two conditions—viz., pulmonary gangrene and putrid bronchitis, in which diseases it has a heavy, pungent smell. At times an odor resembling that of gangrene, and equally unpleasant, is emitted from the sputum of bronchiectasis. Sputum that has been allowed to stand at room-temperature develops a decided odor in seventy-two hours. A sweetish odor is often emitted by the sputum in cases of pulmonary ulceration, bronchitis, and perforating empyema. When tyrosin is present in the sputum of empyema, the odor resembles that of rancid cheese. In case the odor of tyrosin is detected in the sputum, it suggests that some extraneous purulent material has gained access to the bronchial tract.

Consistence and Tenacity.—The density of the sputum will be found to correspond more or less closely to the amount, and to vary from a watery fluid to that of a gelatinous mass. At times, when the sputum is placed in a cylindric glass, it will be found, upon standing, to have separated into strata, the superior of which is covered with a heavy froth or beaded with fine air-bubbles. The sputum immediately, and for some distance below the froth, will be found clear and of a liquid consistence. Below this stratum will be found a layer containing flocculi and particles of mucus; while the lowest stratum, which may at times be the deepest and again the shallowest of these strata, is often composed of a sediment which is purulent or bloody. Sputum may be of a creamy consistence, its color depending largely upon the character of the substances of which it is composed. Liquid sputum is found in edema of the lungs, tuberculous laryngitis, early in pulmonary tuberculosis, perforation of an empyema, or of a diaphragmatic or hepatic abscess; and in pulmonary abscess and gangrene, in all of which diseases the quantity may be large.

The exact causes for the high degree of tenacity often displayed by the sputum remain undetermined. Mucin, while once regarded as an important factor, does not appear to contribute largely to this feature, since highly tenacious sputum is known to occur in cases in which the quantity expectorated during the twenty-four hours is but slight—not exceeding a few cubic centimeters (lobar pneumonia). A highly tenacious sputum was observed by me in a case in which an amebic abscess had ruptured into a bronchus. The tenacity of the sputum is detected by inclining or inverting the receptacle into which it has been expectorated. Tenacious sputum shows little or no tendency to flow, but adheres closely to the surface of the vessel; indeed, the receptacle with its contained sputum may be inverted without danger of spilling. Such a condition is rather characteristic of the sputum of croupous pneumonia before the crisis, and is occasionally seen following an acute attack of asthma, as well as in the early stage of acute bronchitis.

Specific Gravity.—The specific gravity will likewise be found to fluctuate greatly, and will depend directly upon the general character of the sputum. Mucous sputum varies from 1.003 to 1.01; purulent and bloody specimens vary between 1.014 and 1.025; while serous and highly bloody sputum may reach 1.035, but rarely exceed this figure.

Determination.—The specific gravity of the sputum is best determined by placing a quantity of sputum in a bottle the stopper of which is held in position by a wire, to prevent evaporation. The sputum is then cautiously heated to 60° C. (140° F.), at which temperature it is converted into a thin fluid; the specific gravity may be taken in the usual manner (see *Urine*, page 172).

Reaction.—Sputum is of an alkaline reaction.

Color.—The sputum will be found to vary greatly in color from that of a perfectly clear, transparent liquid, through the successive shades—gray, yellow, amber, orange, olive green, red, chocolate, and black. When the expectoration is entirely mucoid, it is colorless and nearly transparent—for example, in the sputum of pulmonary edema (when no blood-cells are present) and in the so-called "albuminous expectoration."

The presence of leukocytes in the sputum renders it opalescent or turbid, according to the number of cells present; and the color, from the same cause, is first white, then yellow, and, finally, of a greenish hue. Green sputum may also be caused by the admixture of bile-pigments, a not uncommon feature of lobar pneumonia when complicated by jaundice, and by the communication of a hepatic abscess with the bronchial tract. The presence of bacteria (Bacillus pyocyaneus) may be accountable for a green color. In cases of amebic abscess, whether hepatic or pulmonary, the sputum resembles anchovy sauce in color, as was observed in my private practice.

Black.—Gray sputum may result from the inhalation of particles of carbon, while the sputum of coal-miners and those residing in the mining districts is often dark and at times black, due to the presence of coal-dust (anthracotic sputum, Plate 30). Particles of iron may give the sputum a yellow or red color.

Bloody Sputum.—Sputum tinged with blood and studded with minute air-bubbles—"rusty sputum"—is quite characteristic of lobar pneumonia; and rusty sputum displaying bloody, punctate areas is to be seen in connection with brown induration of the lung. Blood gives the sputum a red color, which varies in intensity with the amount of this tissue added. It is most often encountered in pulmonary congestion and in ulceration (phthisis). Less frequently hemorrhagic sputum may result from cardiac insufficiency through an embarrassed pulmonary circulation. Whenever the blood is expectorated as soon as it escapes from the vessels, it will be found to be of a bright-red color; but retention in the bronchi favors the development of a brownish-red, dirty-brown, or chocolate-colored sputum. The last is fairly characteristic of pulmonary gangrene, although sputum indistinguishable in color from that of pulmonary gangrene is to be seen in cases in which large tuberculous cavities exist. Bloody and dark-brown sputa are also to be observed in abscess of the lung, as well as in gangrene. In these cases the color probably depends upon the presence of hematoidin, bilirubin, or both. Prune-juice sputum results when blood is retained in an edematous lung; and currant-jelly sputum is regarded as suggestive of a malignant disease of the lung.

Mucous Sputum.—This variety of sputum is clear, sticky, tough, and, during the early stage of bronchitis, scant. In the later stage of bronchitis pus-cells are added which render the sputum more copious and give it a yellowish or a greenish color.

Mucopurulent Sputum.—This is a variety of sputum seen in connection with many forms of pulmonary disease. It is of little clinical value except in cases of pulmonary tuberculosis, in which, in the event of cavity formation, minute, ragged clumps of mucopus which are intimately surrounded by mucus may be seen.

Coin-like masses of sputum (*nummular sputum*, often regarded as characteristic of cavity formation), when first expectorated, float upon the surface; but after a time sink to the bottom of the liquid, where an aggregation of these coin-like bodies forms a dense, purulent sediment. At times such sputum separates into three layers—viz., an upper frothy mucous, a middle serous, and a lower purulent layer. In cases of old cavities the sputum may contain grayish-white masses, or, equally important, round or irregular particles varying in size from that of a pin's point to that of a millet-seed (*caseous particles*). These usually fall through the liquid portion of the sputum and collect at the bottom of the vessel; but should they contain air-bubbles, they then possess a variable degree of buoyancy and float upon the surface or are suspended in the liquid at different depths. Such sputum is fairly suggestive of the second and third stages of tuberculosis.

Similar masses have been found in the sputum of healthy individuals (rarely), and they are occasionally seen in cases of tonsillitis; but these particles differ from those found in the recent sputum of tuberculosis by emitting an unpleasant odor when crushed with a needle, and by not containing tubercle bacilli.

Serous Sputum.—This variety of sputum may be entirely distinct from mucous expectoration. It appears as a watery fluid which is often blood-streaked. It is fairly characteristic of edema of the lungs, and, in the absence of blood-cells, resembles soapy water.

MICROSCOPIC STUDY OF THE SPUTUM.

With the aid of the microscope positive diagnostic evidences are to be obtained from the examination of the sputum. A microscopic study, on the other hand, is often merely suggestive of the existence of certain maladies.

ORGANIZED CONSTITUENTS.

Fibrinous Coagula.—During the course of certain pathologic conditions an exudate is deposited in the smaller bronchi, and after having undergone certain degenerative changes, this exudate results in the formation of a complete cast of a small bronchus and its branching bronchioles, so that a variable area of the lung may be involved. During the act of coughing a small amount of this coagulum, which now forms a complete cast of the bronchus, is dislodged and appears in the sputum as a small, gray, white, reddish, yellow, mahogany, or bloody particle readily detected by the naked eye. Such casts may vary from 2 to 10 cm. in length and from 1 to 3 cm. in thickness.

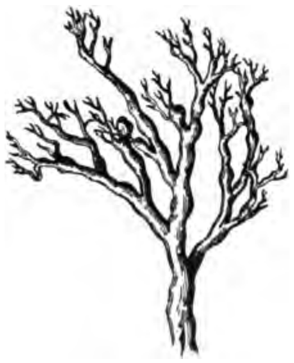


Fig. 180.—Fibrinous bronchial cast.

Detection.—Fibrinous casts may be recognized by placing suspicious particles of the fresh sputum between two slides and making rather firm pressure upon the upper slide. The questionable particles are clearly demonstrable when brought in focus under a two-thirds or one-fourth inch objective (Fig. 180). They appear as fine stems or central trunks from the sides of which several smaller casts may be seen extending. From one extremity of the trunk is seen to extend a number of small, twig-like filaments which divide dichotomously to form casts of the smaller bronchi. The diameters of these branches diminish gradually and terminate in delicate points. In immediate connection with these casts leukocytes, red blood-cells, and epithelial cells may be seen, and sometimes Charcot-Leyden crystals (Fig. 181).

Clinical Significance.—Fibrinous casts have been found in the sputum of fibrinous bronchitis, croupous pneumonia near the time of resolution, and in cases in which there is a diphtheric process in the finer bronchi.

Bronchial Spirals.—These bodies, when present in the sputum, appear to the naked eye much the same as do bronchial casts, except that they are always white or yellowish in color.

Detection.—Treat the sputum as described in the technic given for bronchial casts: at times it will be found necessary to use a high-power objective (one-sixth to one-eighth). Spirals appear as faint, translucent, elongated masses, with indefinite and irregular margins (Fig. 181). A delicate, white, thread-like fiber runs longitudinally through the center of each spiral. This fiber has a tortuous course, and seems to be surrounded by numerous coiled fibrillæ. Leukocytes, epithelial cells, Charcot-Leyden crystals, and, rarely, erythrocytes are seen to be entangled in the mass; while surrounding the fibrous portion there is a faintly granular hazing. The central thread is not discernible in all specimens.

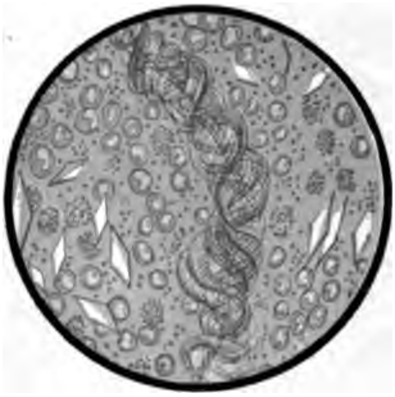


Fig. 181.—Sputum from a case of asthma, showing Curschmann spirals, Charcot-Leyden crystals, leukocytes, and numerous free eosinophile granules; unstained specimen (Jakob).

Clinical Significance.—Spirals are a rather constant finding in the sputum of persons suffering from asthma, both during and after the paroxysmal stage. They are not unusual in cases of croupous pneumonia, acute bronchitis, chronic bronchitis, and I have observed them in the sputum of cases of tuberculosis, valvular heart disease, diphtheria, and typhoid fever. From their mucinous appearance it seems fair to regard them as suggestive of a catarrhal process of the bronchi, yet their true formation remains undecided.

Elastic Fibers.—Fibers of elastic tissue may occur in the sputum singly, in a more or less perfect alveolar arrangement, or, as is most usual, in bundles. They may be demonstrated in the manner described for the detection of "fibrinous coagula," a one-eighth objective being used. The method which I have adopted to pour a quantity of sputum in a rather large glass dish, in ord to secure a thin layer of the sputum, and then to place the dis upon a table or any dark background. The more or less soli particles are thus rendered conspicuous, and are transferred to vessel containing a 10 per cent. solution of sodium hydroxid, by means of a small surgical forceps. Boil the soda solution and its contained sputum until a gelatinous mass results or until the formed particles of sputum are dissolved; then add four times the total quantity of water; place the mixture in a conic glass, and allow it to stand for several hours. Remove the sediment that has collected by a small pipet, and place it in a centrifuge; add water enough to fill the tube and centrifugalize. The sediment obtained by the last process may be studied under a one-sixth or

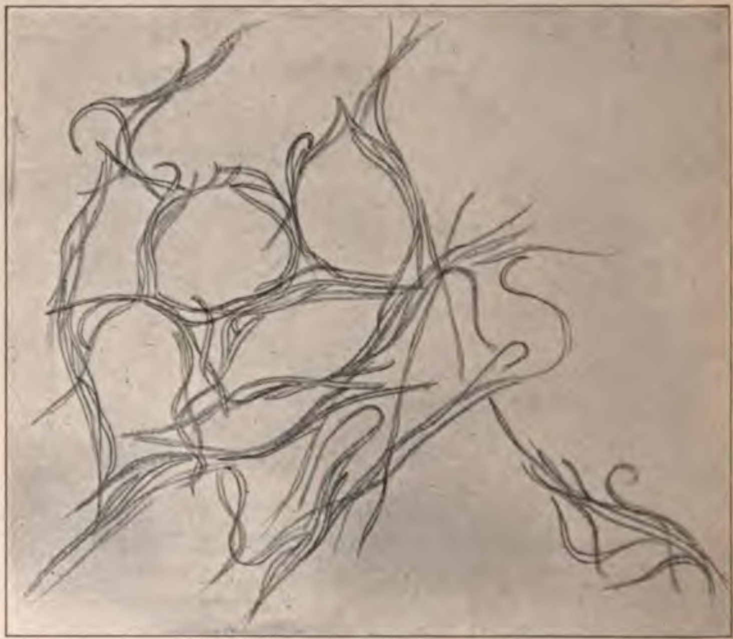


Fig. 182.—Fibers of elastic tissue from sputum in a case of pulmonary tuberculosis observed at Pennsylvania Hospital (obj. B. and L. one-eighth).

one-eighth objective, when elastic fibers, if they are present, are readily detected (Fig. 182). The addition of a drop of Gram's iodin solution to the microscopic specimen facilitates the diagnosis materially.

Caution.—Boiling with sodium-hydroxid solution, when prolonged, causes swelling, and finally a dissolution of the elastic tissue. Attempts to detect elastic tissue without first treating the sputum with sodium-hydroxid solution have not been attended with satisfactory results by the author.

Clinical Significance.—The presence of elastic fibers in the sputum is suggestive of the existence of a highly destructive process in the lung. The true significance of these changes, however, is indicated only when they appear in the so-called alveolar arrangement, as they commonly do in phthisis and as they rarely do in pulmonary gangrene. In the latter condition Traube has suggested that elastic fibers may be destroyed by a ferment. Elastic fibers are rarely found in connection with abscess of the lung, bronchiectasis, and pneumonia.

Caution.—Direct the patient to wash the mouth and brush the teeth carefully before collecting the sputum, to avoid the possible expectoration of elastic tissue from retained food-particles.

ANIMAL PARASITES OF THE LUNG.

Paragonimus Westermanii.—Endemic hemoptysis has thus far been confined to Japan, Formosa, and Corea, although cases have been reported from other eastern countries. On account of the frequency with which the Japanese are infected with this parasite it was named Distomum pulmonale and Distomum ringeri. Drs. Stiles and Hassall have recently recovered this parasite from the lungs of hogs raised in the United States. It has also been found in the lungs of cats and dogs, as well as in the lungs of certain wild animals. On account of the immigration of both Japanese and Chinese and of the temporary residence of United States troops in the tropics the spread of "parasitic hemoptysis" throughout America seems imminent, Dr. A. D. MacKenzie having recently reported a case from Portland, Oregon.

Sputum.—The sputum is, as a rule, tinged with blood, and at irregular intervals attacks of hemoptysis occur which are usually induced by violent exercise. During these attacks the hemorrhage may be severe. In color the sputum may closely resemble that of lobar pneumonia.

Detection.—Place a portion of the bloody sputum upon a slide, apply a cover-glass, and study under a one-fifth or one-eighth objective. The peculiar discoloration of the sputum will now be seen to depend upon the presence of numerous ova or their shells, as well as on the presence of red blood-corpuscles (Plate 29). The ova vary greatly in size and contour, although all will be found distinctly or imperfectly oval, presenting a smooth surface and a double outline. They measure from "80 to 100 μ in length by 40 to 60 μ in breadth" (P. Manson). The parasite may be cultivated in the laboratory by adding water to the sputum and keeping it at a moderate temperature for from four to six weeks. At the end of this time each mature ovum extrudes a ciliated embryo. Pressure of the cover-glass upon the mature ovum may rupture the operculum, when the gyrated embryo escapes and may be seen swimming in the surrounding liquid. This parasite is reddish-brown, rather thick, oval in contour, and measures "8 to 10 cm. in length by 4 to 6 cm. in breadth," and is covered with minute spines.

Bilharzia.—There are numerous valid records of cases in which the ova of Schistosoma hæmatobium have been found in the sputum (see *Urine*, page 295).

Amœba Coli.—This parasite may be discovered in the sputum whenever an amebic abscess is evacuated through the bronchial tract, whether the abscess be pulmonary, hepatic, or subdiaphragmatic. I have observed a case in my private practice* in which the sputum contained many amebæ over a period of four months. About two ounces of semigelatinous sputum were expectorated daily. It was often of a grayish color, streaked with blood, and contained numerous flocculi. Portions of this sputum appeared as though dotted with small droplets of dark-reddish fluid, and this characteristic persisted for weeks after the rupture of the abscess. Such sputum, when placed in a porcelain dish and allowed to stand, developed a marginal, chocolate band; its tenacity was decided and its odor at times sweetish. Microscopically, motile amebæ were studied under an oil-immersion objective (Fig. 145), and occasionally an ameba was observed enveloping a red blood-cell. Epithelial cells arranged in clusters and in radiating columns were also present.

Trichomonades.—Trichomonades (Fig. 146) have been found in the sputum of pulmonary gangrene and in that from pus-cavities. The Balantidium coli (Fig. 147) may invade the respiratory tract and consequently appear in the sputum.

Whooping-cough.—Doechler and Kurloff claim to have observed the protozoön of whooping-cough in the sputum; yet, so far as I know, these observations have not received confirmation.

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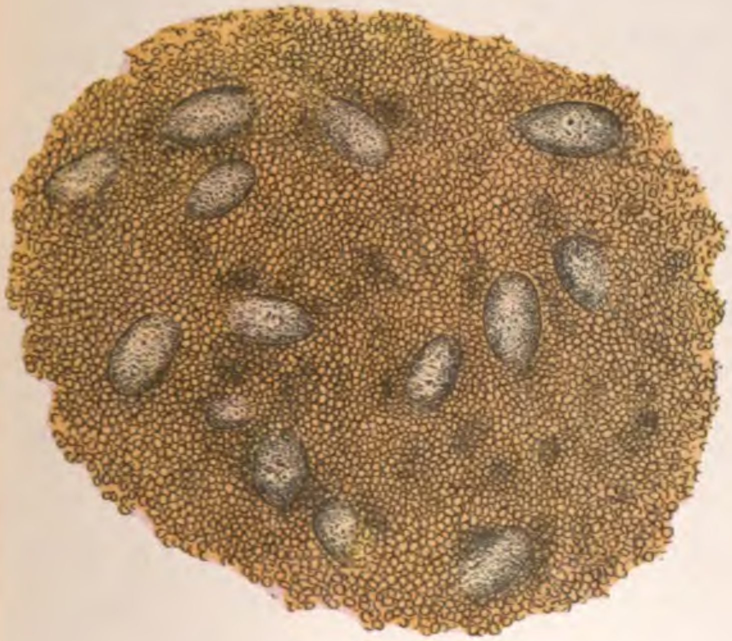
* "Therap. Gaz.," April, 1902.

PLATE 29.

1



2

Paragonimus Westermanii.

1. Six lung-flukes from hogs (natural size and color).

2. Contents of a lung-fluke cyst, containing eggs of the lung-flukes (greatly magnified) (Stiles and Hassall).

Tænia Echinococcus.—Whenever cysts of this parasite rupture into the lung or the air-passages, both hooklets and scolices of the parasite may appear in the sputum; while less frequently small particles of the cyst membrane are expectorated. When these structures (Fig. 122) are found, the evidence points rather clearly to hydatid of the liver, yet hydatids of the lungs are not unknown.

Ascarides.—This parasite or its ova is rarely found in the sputum (see *Feces*, page 414).

Filaria.—The literature contains records of the Filaria sanguinis hominis having been found in the sputum.

FUNGI.

Among the non-pathogenic fungi that are known to appear in the sputum should be mentioned molds and yeasts, which are met with in the sputum of children and which may be seen in the salivary secretions of adults. Sarcinæ are an occasional finding in the sputum of putrid bronchitis. Sarcinæ pulmonale are, as a rule, smaller than Sarcinæ ventriculi (Fig. 138), but larger than the sarcinæ found in the urine.

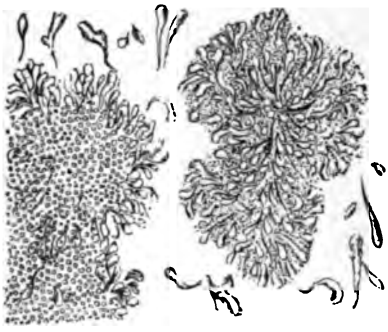


Fig. 183.—Actinomyces (after von Jaksch).

Pathogenic Fungi.—**Actinomycosis** of the respiratory tract is distinguishable from other forms of pulmonary disease through the detection of small granules (Fig. 183) and thread-like particles (mycelia) in the sputum (see page 301).

Aspergillosis.—Aspergillus fumigatus has been found in the sputum of man by foreign observers, and was associated with pneumomycosis. The aspergillus, when present in the sputum, is usually recognized by the detection of many thread-like particles (mycelia) (Plate 19). I have found Aspergillus fumigatus in the sputum of man but once, and in this instance there appeared to be no connection between the fungus and the disease from which the patient was suffering. It is not uncommon to find sputum secondarily infected with Aspergillus niger, and it is difficult to distinguish microscopically between the mycelia of these two fungi.

Dr. M. P. Ravenel has studied the Aspergillus fumigatus from the lung of a cow apparently suffering from pulmonary tuberculosis. The early writers regarded this organism as accountable for the destructive lesions of tuberculosis.

Other fungi, as the **Mucor corymbifer**, are to be encountered in the sputum, and it should be further stated that many different molds may develop in the sputum after it has been exposed to the air of a warm room. As a rule, however, these fungi have no clinical significance, being commonly of extraneous origin. I have seen the yeast fungus in the sputum from tuberculous cavities.

BACTERIA.

Many bacterial species have been found in the sputum, and in a comparatively small number of instances a definite microorganism will be found associated with a certain disease. I have never seen a specimen of sputum in which there was a pure infection with any one bacterium; but, on the other hand, the rule is to find a number of organisms in each sputum. Among the bacteria commonly encountered should be mentioned the pneumococcus, the streptococcus, the staphylococcus, a large diplococcus, the Bacillus typhosus, the Bacillus coli communis, Friedländer's bacillus, the Bacillus diphtheriæ, the bacillus of influenza, the Bacillus tuberculosis, and the streptothrix.

Microscopic Study of the Sputum.—Sputum should be collected in the manner described on page 426, and sent promptly to the laboratory. When the specimen is received, it should be poured into a clean glass dish in order to secure as large a surface as possible. Select from the sputum thus spread out any small caseous or bloody particles that may be apparent, place them upon a microscope slide, and then crush them and spread into a thin layer over the surface. It is my custom to hold the slide within the grasp of a slide forceps (Fig. 184, the author's device for this purpose) while the sputum is being smeared upon its surface. Without removing the slide from the grasp of the forceps pass it three times through the flame of a Bunsen burner to fix the specimen.

Tubercle Bacilli.—**Staining.**—1. Add a few drops of carbolfuchsin to the specimen (5 per cent. carbolic acid, 90 parts; concentrated alcoholic solution of fuchsin, 10 parts), and hold it above the flame until the staining solution begins to steam.

2. Wash in water, holding the forceps in such a manner that the stream strikes the slide near one end and then floods over the specimen.

3. Without drying add to the specimen a few drops of Gab-

bett's methylene-blue solution (methylene-blue, 2 parts; solution of sulphuric acid (25 per cent.), 100 parts), and allow to stand for two minutes, when wash in water and dry over the flame.

4. The specimen may now be mounted in Canada balsam and kept as a permanent specimen, in which case it is well to allow the balsam to harden before studying with an oil-immersion objective.

In routine laboratory work it is my custom to apply the cedar oil directly to the specimen without the application of a cover-glass. In searching for the diplococcus and organisms other than the tubercle bacillus equally satisfactory results are obtained by staining with a 2 per cent. aqueous solution of methylene-blue. Since tubercle bacilli are not uncommonly found in sputum from cases in which the general clinical features are far from suggestive of tuberculosis, it is a safe precaution to stain all sputa for the tubercle bacillus. I have repeatedly seen cases of large tubercular cavities in which it was impossible to stain the tubercle bacillus in the sputum by the above method. In three such cases studied at the Philadelphia Hospital I was privileged to witness autopsies,

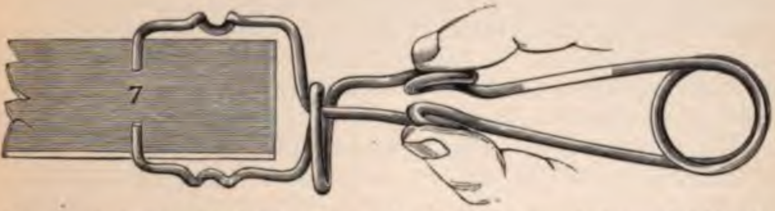


Fig. 184.—Author's combined slide and cover-glass forceps. Forceps in the act of picking the slide from the table.

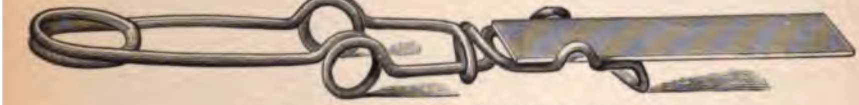


Fig. 185.—Forceps with the slide locked in position.

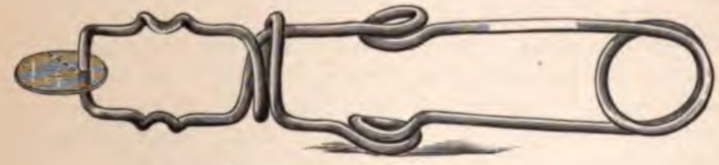


Fig. 186.—As a cover-glass forceps.

during the course of which pus taken directly from the cavities showed many tubercle bacilli after this method of staining.

Caution.—Whenever there are valid reasons for believing that tubercle bacilli are present in the sputum, the following treatment will materially facilitate their detection:

Place the sputum in an ordinary vaselin bottle and tie over its mouth three or four thicknesses of gauze tightly—to prevent dust from entering the bottle. Set the specimen aside for several days and then examine for tubercle bacilli. The processes of decomposition appear to destroy certain substances (probably fats), which in the fresh sputum prevent the tubercle bacilli from taking the stain. A method which I have found most satisfactory for staining such specimens is to smear the caseous particles of the sputum on slides and allow them to dry in the air. The specimen is then fixed either by passing it directly through the flame or by keeping it upon a hot stage (see *Blood*, page 74) for from twenty minutes to one-half hour. Specimens may also be fixed by immersing them in equal parts of absolute alcohol and ether. Specimens fixed by either of these methods should be immersed in a weak solution of fuchsin for from twelve to twenty-four hours (carbolfuchsin, ½ dram; water, 2 ounces).

By these methods it will frequently be found that tubercle bacilli are present in sputum in which it has been impossible to demonstrate them by the rapid method of staining previously described. This staining peculiarity of the tubercle bacillus is most commonly encountered early in tuberculosis, before the clinician is able to detect evidence of pulmonary involvement by the methods of physical diagnosis.

Anilin-gentian-violet.—Gentian-violet forms a most satisfactory stain for the tubercle bacillus, and, while I am not prepared to state that it has advantages over carbolfuchsin, yet in my hands it has been found equally reliable. I began using this stain six years ago in the Philadelphia Hospital, and have found no cause for changing to any other.

Sterling's Stain.—

Gentian-violet	10 gm.
Anilin oil	4 "
Alcohol (95 per cent)	20 "
Distilled water	176 c.c.

Add the anilin oil to the alcohol, and dissolve the gentian-violet in water. Add solution of gentian gradually, shaking vigorously after each addition; filter.

(1) Sterling's solution should be added to the specimen in the manner outlined on page 438, and the slide should then be carried over the flame, and heated to streaming for from one-half to one

minute. (2) Next wash in water to remove all excess of stain. (3) Then treat the specimen with a few drops of a 0.5 per cent. solution of nitric acid until the specimen has apparently given up all its violet color. (4) Wash in water. (5) Counterstain with a few drops of a saturated alcoholic solution of Bismarck-brown for from one-half minute to one minute. (6) Wash in water to remove all stain; dry between layers of filter-paper, and mount the specimen in Canada balsam. Under an oil-immersion objective the tubercle bacilli will be found to be stained a deep violet; all other bacteria will be stained with the Bismarck-brown; the surrounding cells will be stained a light or mahogany brown; the nuclei of the epithelial cells and of the leukocytes will be stained somewhat darker than the protoplasm.

Differentiation.—The tubercle bacillus is to be distinguished from the following organisms, all of which are acid-fast (do not decolorize readily by acids or by alcohol)—viz., Bacillus lepra, smegma bacillus, the butter bacillus of Rabinowitch, and the grass bacillus.

TABLE SETTING FORTH THE TINCTORIAL AND CULTURAL DIFFERENCES OF "ACID-FAST" BACILLI WITH SPECIAL REFERENCE TO THE SOURCES FROM WHICH SUCH BACILLI ARE OBTAINED.

Tubercle Bacillus.	Lepra Bacillus.	Smegma Bacillus.	Moeller's Grass Bacillus and the Butter Bacillus.
1. When stained with carbolfuchsin, does not give up its red color when counterstained for a long time with Gabbett's acid-blue solution.	1. Retains its red color.	1. Retains its red color.	1. Retains its red color.
2. Does not stain well with aqueous solutions of anilin dyes.	2. Stains.	2. Not readily stained.	2. Not readily stained.
3. Does not decolorize when treated with absolute alcohol.	3. Retains its red color, but is decolorized in a shorter time than is the tubercle bacillus.	3. Decolorized. Moeller states that the smegma bacillus is alcohol-proof.	3. Not decolorized.
4. Cultures develop only upon a special medium, and growth does not appear for from ten days to two weeks.	4. Cultivation questionable.	4. Cultivated on special medium questionable, and in this way differs from Bacillus tuberculosis.	4. Grows luxuriantly and with rapidity upon nearly all forms of culture-media when kept at both room and at incubating temperatures.
5. Found in the sputum, saliva, nasal secretions, feces, exudates, transudates, tissues,and rarely in urine, blood, and in milk.	5. Recovered from sloughing surfaces, nasal discharges, in blood recovered by incising a leprous nodule and applying firm pressure; and from the circulating blood.	5. Recovered from smegma of man and from that of the lower animals; also from the skin of the groin, axilla, and from the anus and the urine. It is rarely found in exudates, the tissues, and in sputum.	5. Found about stables, upon hay, and in milk, butter, the feces of cattle, and is to be always looked for in urine and feces of man.

Clinical Significance.—Tubercle bacilli, when found the sputum, furnish conclusive evidence of the existence of tuberculous lesion along the course of the respiratory tract. the case of a small ulceration of the bronchus the number of tuberc bacilli present in the sputum may be very great; thus it is possib to find many tubercle bacilli in the sputum of persons who sho no evidence of pulmonary involvement upon a physical examination; and in this class of cases it is not infrequent to find th patient gaining in weight. Tuberculosis of the larynx is als likely to cause great numbers of tubercle bacilli in the sputum It is the rule to find tubercle bacilli in the sputum of persons in whose lungs there exists an ulcerative process or a cavity of tuberculous origin.

Characteristics of Tubercle Bacilli.—In the sputum of persons suffering from pulmonary tuberculosis of an acute and rapid nature the tubercle bacilli are, as a rule, short, and are less likely to show distinct cross-striations (segmentation) than are the bacilli recovered from the sputum of persons suffering from a chronic type of the disease. In the study of 600 specimens of sputum from nearly 400 different patients it was found that wherever the bacilli were short and failed to show segmentation, the course of the disease was at that time rapid. It was further found that upon sending such patients to the mountains larger segmented bacilli were found in their sputum whenever there was noted a decided amelioration of the symptoms; and that in the sputum of patients returning from the mountains apparently in perfect health large, segmented, and branching forms of bacilli were often detected.

Other Bacilli.—Recent research has demonstrated that a number of bacilli that are not usually regarded as pathogenic for man also possess the property of retaining the red color from carbolfuchsin stain upon being treated with weak solutions of acids and with alcohol (see *Differential Table*, page 441).

Other members of the acid-fast group of bacilli have been recovered from the sputum and in mucus from the respiratory passages. It appears possible that certain of the reports of tubercle bacilli having been detected in both the sputum and the urine are in error; I am, therefore, prompted to insist that a most important clinical feature here outlined is the differentiation between the tubercle bacillus and other acid-fast bacilli. Rosenberger has contributed an admirable monograph upon this subject.*

Diphtheria.—During the course of diphtheria the sputum will commonly be found to contain the diphtheria bacillus, which

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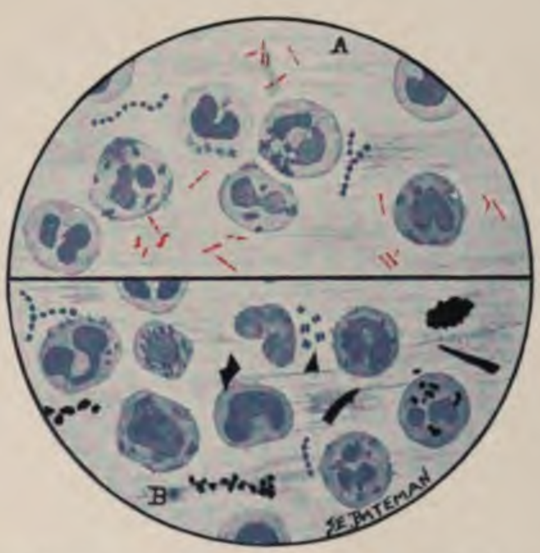
* "Proc. Path. Soc. Phila.," Dec. 10, 1903.

PLATE 30.



A. Sputum showing tubercle bacilli stained with carbolfuchsin and Gabbett's methylene-blue solution (obj. B. and L. one-twelfth oil-immersion).

B. Sputum of anthracosis, showing particles of coal-dust stained with methylene-blue (obj. Spencer one-twelfth oil-immersion).

may be recognized by its characteristic staining and also by the result of cultures made directly from the fresh sputum. Culture studies of sputum, however, are not practical, and constitute a circuitous route to a diagnosis.

Influenza.—The sputum in this disease may contain slender bacilli which stain readily by the ordinary anilin dyes. For their cultivation a special medium is necessary—Löffler's blood-serum will be found to serve well for this purpose, after the surface of the medium has been smeared with fresh blood, since the latter appears to provide certain substances necessary for the development of this organism.

Acute Bronchitis.—Early in acute bronchitis the expectoration is small in amount, transparent, and contains but few cellular elements. When studied microscopically, many desquamated epithelial cells are to be found which represent the various forms common to the bronchial tract. Many of these are originally ciliated, although it is rare to find ciliated epithelial cells in the sputum. Leukocytes are always present, though, as a rule, in small numbers; and red corpuscles may be found, varying in number with the degree of irritation. Later in the course of acute bronchitis the sputum becomes more abundant, opaque, or turbid, and acquires a yellowish or greenish color, depending upon the number of leukocytes or pus-cells added. At this time the number of epithelial cells is found to be greatly lessened.

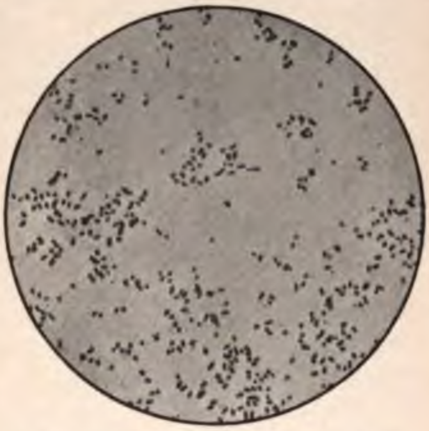


Fig. 187.—Bacillus of influenza, from a gelatin culture (× 1000) (Itzerott and Niemann).

Bacteriology.—Patton * suggests the terms "streptococcus bronchitis and staphylococcus bronchitis." The latter variety of infection is highly liable to chronicity; the sputum contains many staphylococci, which are often collected in such dense aggregations as to prevent their study with a high-power objective.

In the case of streptococcic infection the sputum contains innumerable streptococci, and where this form of infection is mild, it is differentiated from the staphylococcic bronchitis through a microscopic study only.

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* "New York Med. Jour.," Mch. 28, 1903.

The bacteria common to the saliva are also present in the sputum of acute bronchitis, but seldom in sufficient numbers to attract special attention.

Chronic Bronchitis.—It can scarcely be said that there is any sputum characteristic of this affection, since the expectorated material may vary within wide limits during the course of this disease. When the sputum is profuse and, as is sometimes the case, expectorated in mouthfuls, the term *bronchorrhea* is applied. The sputum is, as a rule, yellowish or yellowish-green in color, varying in shade with the number of pus-cells present and the degree or stage of their degeneration.

Many micro-organisms are found in the sputum of chronic bronchitis, among which are to be mentioned streptococci, staphylococci, both large and small bacilli, and bacilli in chains and bundles of chains.

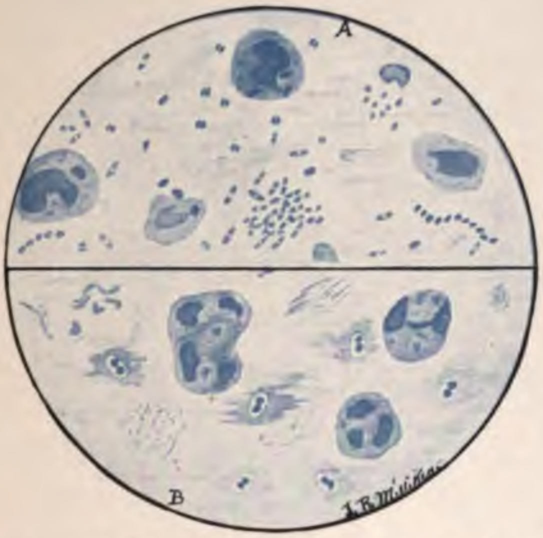
Pneumococcus.—The pneumococcus is a small diplococcus which occurs in the sputum from cases of lobar pneumonia, in which it is often the only organism present and always in great numbers. The diplococcus (Plate 31) is well stained by Gabbett's methylene-blue solution; but its peculiar contour (biscuit formation) is brought out more prominently by Bismarck-brown, so that when the specimen is carefully stained, each coccus is seen to be surrounded by a narrow hyaline space which is bounded by a faint marginal band (capsule). Encapsulated pneumococci are always to be seen with difficulty, and, while the majority of authors attach great importance to this finding, it is my experience that they are commonly met with in the sputum of healthy persons, and that they are only pathologic when present in the sputum in great numbers and in rather dense aggregations. The pneumococcus stains by Gram's method.

Capsular Stain.—The capsule of this organism is rendered apparent by preparing and fixing the specimen as previously outlined. Next apply a 1 per cent. solution of acetic acid for from one to two minutes; remove the acid by washing and dry in the air. Then stain the specimen for from fifteen to forty seconds, with a solution of anilin-gentian-violet, which is made as follows: Place in a test-tube one part of anilin oil and 20 parts of water; shake thoroughly; filter; preserve the filtrate in an amber-colored, glass-stoppered bottle. A saturated alcoholic solution of gentian-violet is now prepared, filtered, and the filtrate similarly preserved. These two solutions should be mixed a short time before use in the following proportions:

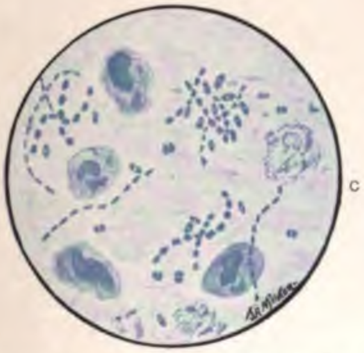
Anilin-oil solution	9 parts
Gentian-violet solution	1 part.

Mix and filter.

PLATE 31.



Sputum containing plague bacilli and diplococci, stained with an aqueous
n of methylene-blue (obj. B. and L. one-twelfth oil-immersion).
Sputum showing pneumococci.



Scraping from tonsil stained with Löffler's methylene-blue, showing diph-
bacilli, diplococci, and streptococci (obj. Spencer one-twelfth oil-immersion).

Bacillus of Friedländer.—The pneumobacillus appears in great numbers in the sputum of persons suffering from lobar pneumonia whenever the disease is due to this parasite (Fig. 188). It stains readily by the same methods as does the pneumococcus, showing also a capsule formation. Friedländer's bacillus may enter the blood, and is to be found in the pus from abscesses and from inflamed joints complicating an attack of Friedländer's pneumonia or developing as sequelæ of this disease.

A number of micro-organisms are usually present in such sputum; the longer the sputum is retained in the bronchi, the more numerous are the bacteria. A few red blood-cells may be found, and epithelial cells are also present, although, as a rule, less plentiful than in the early stage of acute bronchitis. Epithelial cells, showing evidence of fatty and of myeloid degeneration, are also met with and are probably derived from the alveoli of the lung.

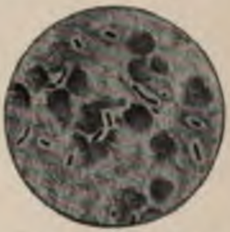


Fig. 188.—Friedländer's bacillus in pus from pulmonary abscess (obj. Spencer one-twelfth).

Bronchopneumonia.—Bronchopneumonia is not placarded by a characteristic sputum. The sputum may contain different bacteria, among which are the pneumococcus, Streptococcus pyogenes, Staphylococcus pyogenes, bacillus of Friedländer, Bacillus pyocyaneus, diphtheria bacillus, Bacillus typhosus, Micrococcus tetragenus, and meningococcus. It is the rule to find more than one bacterium present in the sputum of catarrhal pneumonia.

Bronchial Asthma.—At the beginning of an attack of asthma the sputum is scanty, and may be clear, grayish, or rarely red, owing to the presence of red blood-cells. It is always frothy. Asthmatic sputum is usually characterized by the presence of small, yellowish or grayish particles, "bronchial spirals" (see *Spirals*, page 433), Charcot-Leyden crystals (Fig. 189), and leukocytes. Many of the leukocytes show a special affinity for basic dyes, while the majority of them are decidedly eosinophilic (Fig. 190).

Pulmonary Abscess.—The fresh sputum from an abscess of the lung does not emit a decided odor. When subjected to microscopic study, it will be found to consist principally of pus-cells, hematoidin crystals, fragments of lung tissue, and numerous crystals, among which cholesterin and those of the fatty acids are most common. Fibers of elastic tissue are not unusual findings in the sputum of abscess (see *Elastic Tissue*, page 434). Hepatic and other forms of amebic abscesses have been discussed under *Animal Parasites* (Amœba, page 388).

Pulmonary Gangrene and Putrid Bronchitis.—In these conditions the sputum presents practically the same characteristics, so that it is scarcely practicable to make a differential diagnosis from the microscopic examination of the sputum. They are distinguished one from another by microscopic study only, by the finding of particles of pulmonary tissue derived from the parenchyma of the lungs in the case of gangrene. The sputum of gangrene deposits a rather characteristic sediment when placed in a conic glass and allowed to stand for several hours. At the end of that time it will be found to have separated into strata: the inferior stratum is grayish-yellow or brown and contains pus, small particles of a brown or greenish tint which vary in size from that of a millet-seed to that of a kernel of corn, and, less often, particles

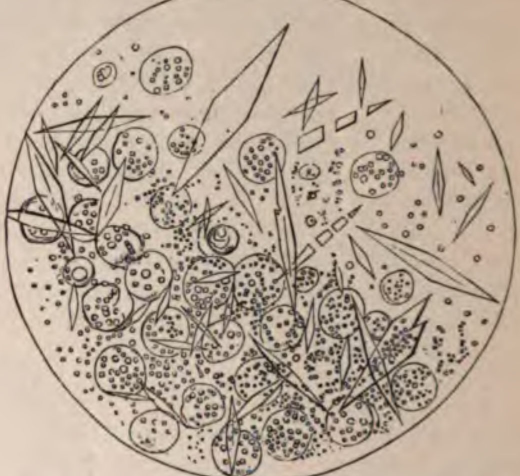


Fig. 189.—Charcot-Leyden's asthma-crystals (after Riegel).

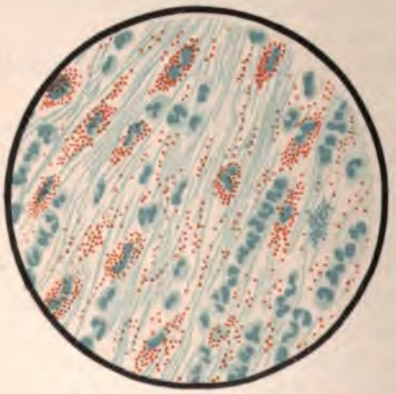


Fig. 190.—Sputum from a case of asthma, showing leukocytes, some containing eosinophile granules; free eosinophile granules; and micrococci; stained with eosin and methylene-blue (Jakob).

of lung tissue. On microscopic examination this sediment will be found to contain triple phosphate crystals, leucin and tyrosin, hematoidin, and, at times, other crystals of a questionable nature. Pus-cells and leukocytes are plentiful, and the masses detected by the naked eye are found to be composed principally of pigment. Elastic fibers, oil-droplets, crystals of the fatty acids, and bacteria are among the usual findings. The Leptothrix pulmonalis (Fig. 193) deserves special mention. It is recognized, in addition to its chain-like formation, by the fact that it is colored violet or blue by Lugol's solution. The detection of elastic fibers (alveolar arrangement, Fig. 182) serves as the only positive differential point between putrid bronchitis and pulmonary gangrene; but in my own experience this tissue is seldom found in the latter condition, of which it is said to be characteristic.

The middle stratum is transparent, and in it are suspended particles of mucus; while the superior stratum is usually of a dirty-yellow color and is covered with a decided froth.

Pulmonary Tuberculosis.—The sputum from a case of pulmonary tuberculosis displays nothing characteristic, although when complications exist, the sputum may be placarded by certain peculiarities.

Incipient Phthisis.—Here the sputum is usually scanty, grayish-yellow or whitish in color, frothy, and tenacious. The bulk of the day's product is ejected in the morning, and even this may not exceed a few cubic centimeters. Hand in hand with the progressing pulmonary involvement the sputum will be found to increase in quantity. After cavity formation the sputum displays coin-like masses of yellowish or grayish mucopus (see *Nummular Sputum*, page 431).

Microscopically the tubercle bacillus is to be detected (see page 438), but red cells are not usual, and in other respects the sputum of tuberculosis is practically the same as that of chronic bronchitis.

When the admixture of blood is large, the entire sputum may form a rather dense clot; but at other times the presence of a small quantity of blood controls the color of the sputum. Dark and blackish sputum is occasionally seen (see *Color*, page 430), and, in cases in which the hemorrhage is very large, it is often difficult to determine whether the blood is of pulmonary, gastric, pharyngeal, or aneurysmal origin. In this connection I am prompted to offer the following differential table:

Pulmonary Hemorrhage.	Gastric Hemorrhage.
1. Evidence of preëxisting pulmonary disease.	1. Referable to the throat, stomach, liver, or heart.
2. Preceded by thoracic oppressions and a saline taste.	2. Preceded by giddiness, faintness, and nausea.
3. Blood ejected by coughing when hemorrhage is small.	3. Blood ejected by vomiting or by clearing of the throat.
4. In profuse hemorrhage and when ejected immediately blood arterial in color.	4. Blood of gastric origin dark, as a rule; blood of pharyngeal origin, bright red.
5. Alkaline reaction.	5. Gastric blood acid, pharyngeal blood alkaline, in reaction.
6. Blood mixed with particles of mucopus.	6. May contain undigested food.
7. A pronounced beaded froth.	7. Froth less marked.
8. Microscopically tubercle bacilli and elastic fibers.	8. Microscopically, Sarcinæ ventriculi, starch-granules, particles of food, and, in the case of carcinoma, large bacilli (Oppler-Boas, page 350), and rarely carcinoma cells.

The color of the sputum may be so modified by retention of the blood in an edematous lung that it is unreliable as a distinctive feature between gastric and pulmonary hemorrhages. In such cases either form of hemorrhage may result from cardiac insufficiency.

Edema of the Lung.—The sputum associated with this condition is always profuse, watery, and presents a heavy froth. In color it will be found to vary from a translucent fluid to that of a dirty reddish or brown liquid. The darkening results from the admixture of blood-cells and the presence of methemoglobin. On account of the fact that such sputum is the result of transudates it is found to be rich in serum-albumin; while microscopically it contains red and white blood-cells in variable numbers; although the red cells are apparently too few to account for the decided color which is often present.

Pneumonia.—The characteristic sputum of this disease is to be seen during the early stage of consolidation in lobar pneumonia, at which time it is scanty and tinged with blood (rusty). Such sputum is highly tenacious, and shows no tendency to flow from the side of the receptacle (see *Tenacity*, page 429). In this stage of the disease the microscope shows the presence of red corpuscles, upon which the rusty color may depend; but in certain instances very few red cells are present, and then the color depends upon the presence of hemoglobin which has been separated from the corpuscles. A varying number of leukocytes is always present, and upon staining, many of these cells are seen to be eosinophilic, a condition more common in the sputum of bronchial asthma (page 445). Alveolar epithelial cells are, as a rule, present, and many of these are studded with granules of pigment, oil-globules (fat), and myelin areas. Larger epithelial cells may be seen, which are probably derived from the larger bronchial tubes or from the mouth. Fibrinous casts may be detected by the naked eye (see *Fibrinous Casts*, page 432), and, in the event of such complications as pulmonary abscess or gangrene, the sputum will present the characteristics of these conditions (see *Abscess*, *Gangrene*).

Pneumococcus.—The pneumococcus (described on page 444), which is present in great numbers during the course of this disease, and particularly in the early stage of hepatization, will be found massed together in rather dense aggregations throughout the field. From this arrangement, unless an oil-immersion objective is employed, the organisms are likely to be mistaken for staphylococci. After resolution has begun (third stage), the sputum of pneumonia cannot be said to be characteristic, but simulates that of a catarrhal bronchitis.

Organic Heart Disease.—In cases of organic heart disease it is not uncommon to find blood-stained sputum: but such sputum may be readily distinguished from the sputum of pneumonia, since it will be found to contain but few diplococci. Alveolar epithelial cells in the bodies of which small brownish or brownish-red granules of hematoidin are deposited—"heart-disease cells"—are a rare finding. In the event of a hemorrhagic infarct having occurred in the lung, the sputum is at first red in color, but later becomes dark and resembles the condition seen when blood is retained in the bronchi before it is ejected.

Pneumoconiosis.—Under this caption it has been deemed of value to describe briefly the sputum seen in cases of anthracosis, chalicosis, stycosis, and siderosis.

Anthracosis.—This condition is rather common in coal-miners and in persons residing in a district in which particles of coal-dust and carbon are constantly floating in the air. Early during this condition, when the lung tissue has not become seriously embarrassed by the deposit of such dust, the sputum is slight in amount, and probably the only expectoration of the day occurs upon rising or after a meal. Later, when a decided bronchitis is present as the result of the foreign material in the mucous membrane of the finer bronchi, the sputum becomes more copious and may, at times, contain large mucopurulent granules, spirals, and rarely fibrinous casts. At times during the course of anthracosis the sputum will present to the naked eye a variable degree of browning or an irregular distribution of black pigment. Such sputum should be stained for the tubercle bacillus. It has been my privilege to detect this organism in more than one hundred specimens of anthracotic sputum. The characteristic finding, however, is that of small particles of coal-dust, which are readily detected under a one-fifth objective; and when viewed under an oil-immersion objective, these masses cannot be mistaken (Plate 30). The other microscopic findings are those common to chronic bronchitis and to asthma.

It is not uncommon to detect particles of carbon in the sputum of persons who smoke to excess; but these particles differ from those present in the sputum of coal-miners by the facts that they are grayish or brownish in color, and that they do not present clear-cut surfaces, a characteristic feature of the particles of coal-dust seen in anthracotic sputum.

Chalicosis.—Chalicosis is a condition in which the sputum contains small particles of the silicates.

Stycosis.—Stycosis is a condition in which the sputum is seen to contain particles of lime, plaster-of-Paris, etc. The condition has been described at length by A. Robin. Like anthracosis, stycosis is accompanied by cough and free expectoration. *Stonemason's lung* results from the inhalation of lime-dust and particles of stone, the presence of which may be detected in the sputum by its chemic reaction. Particles of lime are also to be found within the alveolar epithelium and in the leukocytes. Other characteristics of the sputum are practically those of chronic bronchitis.

Siderosis.—The sputum in cases of siderosis is, as a rule, brown or black in color, while in other respects it resembles that of chronic bronchitis. Microscopically many alveolar epithelial cells and leukocytes are to be seen, both of which are often studded with a variable amount of reddish pigment. Sputum containing iron, when treated with ammonium sulphid, develops a black color, due to the formation of iron sulphid. To another portion of a highly colored sputum supposed to contain iron add hydrochloric acid and potassium ferrocyanid; the presence of iron is indicated by the development of a Prussian-blue color.

CHEMIC STUDY OF THE SPUTUM.

ORGANIC SUBSTANCES.

The sputum will be found to contain certain organic substances other than those previously described, among which albumins, ferments, glycogen, and volatile fats deserve brief mention.

Proteids.—Among the proteids commonly found in the sputum serum-albumin, a liberal quantity of mucin, and nuclein are normal constituents. In the expectoration from cases of croupous pneumonia, purulent bronchitis, abscess, draining through the bronchial tract, and cases in which pus-cells have gained access into the sputum peptones will be found as a constant constituent.

Detection. — Serum-albumin is best detected as follows: treat the sputum with dilute acetic acid, filter, and test the filtrate as described under the Chapter on *Urine* (page 206).

Excess.—A large amount of serum-albumin will be found in the sputum of pulmonary edema, and in allied conditions in which the albuminous constituents of the blood are ejected with the sputum.

Ferments.—In pulmonary gangrene and in putrid bronchitis the sputum has been found by Stolnikow to contain a ferment which, in certain respects, resembles the pancreatic ferments. It is insoluble in glycerin, and may, therefore, be extracted from the sputum by the aid of this substance. This ferment, when present in the sputum, is probably suggestive of a highly destructive process in the lung.

Glycogen.—Glycogen may be present in the sputum, and its detection is accomplished by the methods suggested by Williamson (see *Blood*, page 68).

Fatty Acids.—In order to obtain the volatile fatty acids present in the sputum dilute with water, acidify with phosphoric acid, and distil. Examine the distillate for the presence of volatile fatty acids according to the technic described in the chapter on Feces. Acetic, butyric, and carbonic acids are occasionally found. The non-volatile fatty acids of the sputum are detected by first acidifying the sputum with phosphoric acid and extracting with ether. Repeatedly shake this ethereal extract with an aqueous solution of sodium carbonate; by this means the acids are converted into their respective salts, but remain suspended in the solution. Remove the ether by siphoning, and obtain the fats after the ether has been evaporated. Indol, phenol, and skatol appear in the sputum of pulmonary gangrene.

CHAPTER VI.

BUCCAL SECRETION.

THE salivary secretion is properly classed among the mixed secretions, since it is derived in part from the salivary glands (parotid, submaxillary, and sublingual) and in part from the ducts of the mucous glands within the mouth, all of which open into the buccal cavity. The saliva is subject to great chemic variations, since the disproportionate function of any one set of these glands or all of them materially alters its chemic composition.

Collection.—Direct the patient to wash the mouth, brushing the teeth carefully with a solution of sodium bicarbonate, and afterward rinsing the mouth thoroughly with cold water. Touch the inner surface of the cheek or the edge of the tongue with a glass rod that has been dipped into dilute acetic acid or dilute hydrochloric acid. As soon as the acid touches the mucous membrane the saliva will be seen pouring into the buccal cavity from a number of points. The patient may be allowed to expectorate the saliva directly into an earthen receptacle, or the secretion may be siphoned into a convenient receptacle as follows: A glass tube may be arranged with a bulbous expansion at one end through which there are many small openings. This portion of the tube is placed beneath the tongue, while the remainder of the tube, bent at a proper angle, empties into the receptacle.

PHYSICAL PROPERTIES.

Normal fresh saliva is colorless or may present a slight bluish hue; it usually is fluid in consistence, though at times semifluid, tenacious, and stringy. Upon standing in a conic glass the saliva separates into two layers: an inferior cloudy or turbid stratum, which contains the majority of the morphologic elements, and a superior stratum, usually the deeper and clearer, which is often covered with a beaded froth.

Quantity.—The quantity of saliva secreted a day will be found to vary between 200 and 1500 gm. (6 to 48 fl. oz.).

Reaction and Specific Gravity.—Normal saliva is alkaline in reaction and has a specific gravity of from 1.002 to 1.006.

CHEMISTRY.

On account of the disproportionate activity of the different glands it is with difficulty that any clinical observations of value can be made in this direction, except in the saliva of a few special conditions (ptyalism and opium-poisoning). Traces of albumin, mucin, and, less often, potassium salts are to be found in this secretion. In addition, a ferment, capable of changing starch into sugar, and other salts are normally present. When it is possible to obtain the secretion of the parotid gland, such saliva has been found to contain carbonic acid, nitrogen, and oxygen. In health the buccal secretion concerns us, as clinicians, but little. During diseased states this secretion is diminished to such an extent that its study is impracticable, except in cases of ptyalism, in which the secretion is profuse. For chemic analysis it is possible to collect pure saliva, but this is best approximated by directing the patient to rinse the mouth carefully after each meal and to expectorate all secretions that accumulate in the mouth into a clean vessel. In this way the twenty-four-hour product may be collected, and its specific gravity, reaction, consistence, etc., ascertained after the manner described for the study of urine.

Sulphocyanids.—Place a few cubic centimeters of saliva in a test-tube and treat with a solution of ferric chlorid. When sulphocyanids are present, a bright-red color develops which is unaffected by the application of heat or by the addition of acids. Should no red color appear, place 50 to 100 c.c. of saliva in a casserole and evaporate over a water-bath to a few cubic centimeters. Repeat the test as above outlined. Sulphocyanids also give an emerald-green color when treated with cupric sulphate; precipitate the saliva with alcohol, filter, evaporate the filtrate to dryness over a water-bath, and to this residue add a few cubic centimeters of water.

Opium Poisoning.—The saliva of persons poisoned with opium will give a cherry-red color when treated with ferric chlorid. This reaction depends upon the presence of meconic acid, and the color is not altered upon the addition of mercuric chlorid. The reaction (red color) due to sulphocyanids will be found to disappear when mercuric chlorid is added.

Sugar.—Sugar may also be obtained from the saliva after the manner described in the Chapter on *Urine* (page 224), observing the above precautions.

Diastatic Ferment.—The presence of a diastatic ferment may be detected as follows: Mix 5 c.c. of saliva with 50 c.c. of starch water, heat over a water-bath at a temperature of 40° C. (104° F.)

for one hour, at which time this mixture will be found to give definite reactions for grape-sugar should an amylolytic ferment be present.

Control.—The mixed saliva should be tested for sugar before the process of heating is begun.

Nitrites.—Nitrites of the saliva are demonstrated by adding a mixture of starch paste, dilute sulphuric acid, and potassium iodid. In the presence of nitrites an intense blue color develops.

Greiss' Test.—Dilute a quantity of saliva with five times its volume of water, add a few drops of sulphuric acid and meta-diamidobenzol, and heat to 63° or 65° C. (145.4° to 149° F.). An intense yellow color is indicative of nitrites.

MICROSCOPIC APPEARANCE.

When examined under the microscope the saliva will be seen to contain a number of morphologic elements which are found to vary greatly both in health and in disease.

The salivary corpuscles are bodies which resemble the white blood-cells in many respects, but which differ from these bodies in the following particulars: they are larger; their protoplasm is usually more granular, and they do not possess the clear-cut margin so characteristic of the leukocyte. Red blood-cells may be found in pathologic saliva in connection with a variable number of leukocytes.

Epithelium.—Epithelial cells are found in both pathologic and normal saliva. Since these cells are derived, as a rule, from the mucous membrane of the mouth and tongue, they vary greatly in number in health, and a variety of forms and sizes are always present. The morphologic characters, however, are governed by the respective layer of the mucous membrane from which the cells are derived. There is nothing characteristic of the epithelium ejected with the saliva, and when stained, they may show areas of degeneration; both the cell-bodies and the nuclei often stain abnormally.

Bacteriology.—Molds are not a common finding in the buccal secretion. Fission-fungi are occasionally met with in healthy saliva, occurring in dense aggregations in various portions of the microscopic field. They are stained reddish with a solution of iodin and potassium iodid. The ray-fungus may be found in the saliva in cases of actinomycosis (page 458), and the thrush fungus is also an occasional finding (page 457). In one case in which favus involved the tonsils I recovered the fungus from the saliva (Fig. 192).

The saliva may contain the bacteria of an infectious disease during an attack of that disease; for example, pneumonia and typhoid fever. Among other organisms found in the saliva deserving special mention are: Leptothrix buccalis, Spirochæta dentium, Vibrio buccalis, micrococcus of septic sputum, Micrococcus tetragenus, a bacillus found in connection with decaying teeth, Staphylococcus pyogenes albus, Staphylococcus pyogenes aureus, Streptococcus pyogenes, tubercle bacillus, Bacillus diphtheriæ, Bacillus pestis, Bacillus lepræ, the fusiform bacillus, the spirillum of Vincent, the pneumococcus, a large diplococcus, meningococcus, and the diplococcus of Class (scarlatina). It is not infrequent to find bacilli in the saliva of healthy persons which in morphologic and cultural characteristics resemble closely the bacillus of diphtheria. In fact, at times it is impossible to distinguish between this pseudodiphtheria bacillus and the true Klebs-Löffler bacillus, except by the aid of inoculations. Cultures from the saliva are seldom of practical value; but when such studies are undertaken, the inoculation should be made from material obtained directly from the mucous surface of the buccal cavity.

BUCCAL SECRETION IN DISEASE.

For clinical significance of buccal secretion in disease, see p. 534.

Catarrhal Stomatitis.—Catarrhal stomatitis is a condition which is characterized by an increased quantity of saliva which is acid in reaction. Microscopically many epithelial cells, leukocytes, and, at times, red blood-cells may be found in the fluid.

Should the condition progress to ulceration, the saliva becomes fetid, brown or blackish in color, and alkaline in reaction. At this stage of the disease the microscope shows many red blood-cells, leukocytes, tissue débris, and, at times, many fungi and bacteria.

Ulcerative Stomatitis with Angina.—Of late the records of a number of cases of ulcerative stomatitis with angina have appeared in the literature. In this disease the saliva was found to contain the "fusiform bacillus and spirillum of Vincent." The majority of these reports have appeared in foreign periodicals, principally those of France and Germany; while the English literature shows few such records. Among the last should be mentioned the researches of Emil Mayer; * also a report of two cases by Fisher.† Vincent ‡ first called attention to the fact that in certain forms of ulcerative angina fusiform bacilli and spirilla were to be found in the saliva. In 1897 the same author reported the studies of 14 additional cases in which these organisms were found.* In 1897 Bernheim published his findings in 30 cases of stomatitis with angina, although he did not regard the bacillus of Vincent as the etiologic factor in his cases. Niclot and Marotte have made an exhaustive bacteriologic study of the saliva in this condition, and from their conclusions I have made the following abstract, which embraces the characteristics of these organisms.

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* "Amer. Jour. Med. Sci.," Feb., 1903.

† *Ibid.*, Sept., 1903.

‡ "Annal. de l'Institut Pasteur," 1896.

Bacillus.—The fusiform bacillus (Fig. 191) is expanded at its center and displays pointed extremities; it is, as a rule, straight, but at times is slightly curved or bent upon itself. It measures 6 to 12 μ in length. In the smeared saliva it often appears in rather dense aggregations, sometimes resembling diplococci, and at other times resting at right angles to each other. The organisms are slightly motile. They stain readily with the ordinary basic dyes; but, as a rule, they are nearly all decolorized by Gram's method, and those organisms which resist the decolorizing process are usually involution forms, and are often found to show vacuolation and granulation. Spore-formation has not been determined.

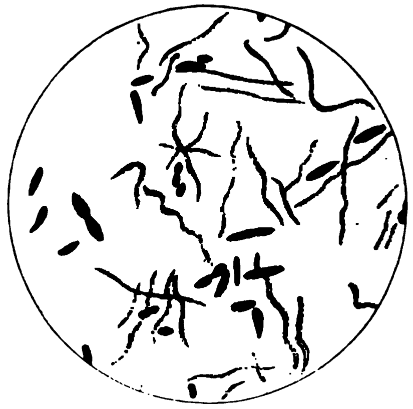

Fig. 191.—Bacillus and spirillum of Vincent, from ulcer in case of ulcerative stomatitis. Stained by carbolfuchsin (obj. B. and L. one-twelfth oil-immersion; × 1000).

Spirillum.—The spirillum (Fig. 191) varies in its dimensions, but is, as a rule, long and of uniform diameter throughout. It occurs singly or in clusters, and less often in dense masses. It is motile, and the motility may persist for some hours, although it diminishes upon exposure to the air or at a low temperature. This organism is stained by the ordinary basic dyes, but is readily decolorized by Gram's method. In deep ulcerations both bacilli and spirilla are present, but where the ulcerative process is superficial, only fusiform bacilli are found. This rule, however, does not always hold true. Vincent's bacillus and spirillum may be found associated with the streptococcus, the pneumococcus, the Klebs-Löffler bacillus, and the colon bacillus in the secretion from superficial ulcerations, but should the secretion be taken from a deeper source, it is most likely to show only bacilli and spirilla.

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* "Bul. et Mem. Soc. Med. des Hôp.," Mch., 1898.

Thus far cultivation and inoculation have given negative results.

Gonorrheal Stomatitis.—In a few instances stomatitis has been found to result from infection with the gonococcus, and while chancroidal infection of the mouth is occasionally seen, both these conditions must be extremely uncommon in America. In several cases of stomatitis, supposedly gonorrheal, I found, by cultural methods, that the organism in question was not the gonococcus, but an intracellular diplococcus which did not stain by Gram's method.

Thrush.—Thrush is not an uncommon finding in the buccal secretions of children, while occasionally it may be encountered in the saliva of adults. In the latter case it is found in persons whose vitality is greatly reduced—*e. g.*, those suffering from tuberculosis. In this disease the saliva is often acid in reaction, although the thrush fungus (Oïdium albicans) may develop in an alkaline medium, yet it, like other fungi, will be found to develop well in acid media.

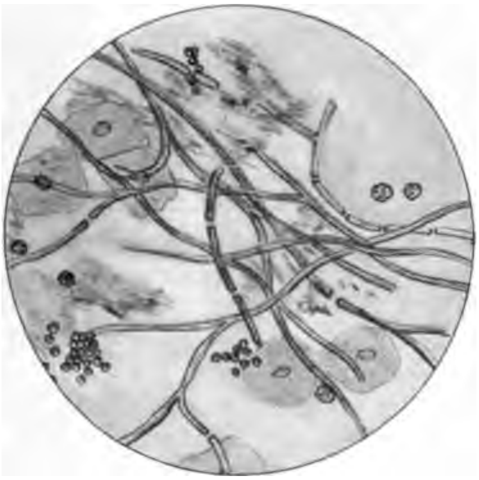

Fig. 192.—Thrush fungus, epithelial cells, and leukocytes from a child suffering from ulcerative stomatitis (obj. Spencer one-sixth).

Detection.—Remove a small portion of the white patch which appears on the mucous membrane, smear thinly upon a slide, and examine under a two-thirds or one-sixth objective, when the mycelia and the spores of this fungus will be readily detected (Fig. 192). Leukocytes, tissue débris, red cells, and branching, ribbon-like segments of the fungus are constant findings. Well-defined oval cells displaying one or more nuclei are also present, and the desquamated epithelial cells are commonly found in clusters of four to ten each. Later in the course of the disease the initial patches may coalesce, but this does not influence materially the characteristic features of the fungus. The mycelial threads of the thrush fungus are well stained by anilin-gentian-violet and by an aqueous solution of methylene-blue. Staining shows that each segment of the ribbon-like bodies contains a highly refractive, especially well-stained, portion imbedded near one extremity. These bodies (spores?) may be situated at each extremity of the segment; they are at times finely granular and oval in outline. Staining is by no means necessary for the study of this fungus, and satisfactory results are to be obtained by adding glycerin to the exudate and studying as previously outlined.

Actinomycosis.—Pus containing the actinomyces is at times discharged with the buccal secretion. J. Wright reports a case of infection of the tonsil.*

COATING OF THE TONGUE.

The coating of the tongue when subjected to a microscopic examination may, in certain febrile conditions, contain great numbers of various forms of epithelial cells, and dark cellular bodies, doubtless degenerated epithelium. Black coating is probably dependent upon a formation of pigmented papillæ, while a white coating is normal for children and will be found to accompany gastro-intestinal conditions in the adult. In these conditions epithelium, salivary corpuscles, and a few fungi may be present.

TARTAR OF THE TEETH.

Chemically, the tartar on the teeth is composed of calcium salts; but, as a rule, it is found to be principally composed of calcium salts because of the greater solubility of the potassium and sodium salts. Either potassium or calcium may exist as urates, and in certain instances sodium urate forms the principal ingredient of the so-called tartar and scales. The above deductions are taken from a series of analyses made by Dr. James C. Attix.

The urates preponderate in the tartar collected from the teeth of persons known to be of a rheumatic or uric-acid diathesis.

Theory.—The secretions of the parotid, sublingual, and submaxillary glands are known to be rich in mucin, and contain CO_2 prior to being poured into the oral cavity, when these secretions are under slight pressure. Upon escaping into the mouth this pressure is released, CO_2 escapes, and thereupon the precipitation of the contained salts is favored. This action is enhanced further in the presence of the lactic-acid fermentation. A slight acid reaction of the secretions of the mouth favors this process, because mucin is thereby made thick and viscid, thus entangling the minute particles which go to make up these concretions.

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* "Amer. Jour. Med. Sciences," July, 1904, p. 74.

Characteristics.—These tartars are, as a rule, quite solid and granular. When the urates predominate in them, they have a yellowish hue, although they may at times be stained from the use of tobacco, etc. The color and consistence of the tartar deposited upon the teeth of those suffering from a general pericementitis, as is seen in marked anemias, are variable: at times, light, and at other times dark; always offensive in odor and rich in particles of food and in lactic acid.

Bacteriology.—Tartar from the teeth smeared out thinly upon a slide, when stained with carbolfuchsin or methylene-blue, will be found composed principally of long bacilli arranged in chains, and these chains in ribbon-like bundles (Leptothrix buccalis). These organisms are stained a bluish red by a solution of iodin and potassium iodid. It has been at times regarded as a cause of dental caries, but many authorities claim that other organisms contribute largely toward this process. Streptococci and staphylococci are also to be obtained from the tartar collected about the teeth, but these organisms are not usually pathogenic.

Pernicious Anemia.—Of recent years considerable attention has been given to the subject of dental caries, the tartar of the teeth, and the existence of a high grade of anemia. In fourteen cases of pernicious anemia in which the blood counts gave the number of red cells below 1,250,000 per cubic millimeter I found that the teeth were in bad condition in many, so that I was often able to recover pathogenic bacteria, cocci, and bacilli from this source. Again, in a rather large percentage of my cases, the teeth were found to be in perfect condition, and out of three cases studied through the courtesy of Dr. L. Webster Fox, two showed that the teeth had never required the attention of the dentist and were in perfect condition, while the third showed the teeth to be in a healthy state.

TONSILLAR MEMBRANE.

During the course of **tonsillitis** a membrane may be removed from the tonsil. When stained, this membrane is often found to contain streptococci, diplococci, staphylococci, or a combination of all in conjunction with bacilli. In scarlet fever Class claims to have found a large diplococcus in a high percentage of his cases. In catarrhal tonsillitis the secretions are liable to show many bacteria.

Diphtheria.—In cases of suspected diphtheria it is often possible to diagnose the disease from a microscopic study of the false membrane. It is my practice to secure a small portion of the membrane by means of a forceps, and to smear it thinly upon a slide. The specimen is then fixed by heat, stained for three minutes with Löffler's methylene-blue solution, washed in water, dried, and studied under an oil-immersion objective. In the vast majority of instances it will be possible to detect typical diphtheria bacilli in such secretions. I have employed this method and have at the same time made cultures as control tests and examined them after they had been kept for twenty-four hours in the incubator. In more than 70 per cent. of the cases it was possible to recognize the diphtheria bacillus beyond question of a doubt in the stained specimen, and in every instance these findings were confirmed by cultural studies.

Caution.—It will often be found that the false membrane removed from the tonsil contains only streptococci, and rarely it will be found to contain bacilli. Still the bleeding surface which results from the removal of such a membrane appears to the naked eye identical with that of diphtheria. Again, the membrane may not leave a bleeding surface after removal, and yet the microscope will show Klebs-Löffler bacilli in great numbers. The pseudodiphtheria bacillus, when present, cannot be distinguished microscopically from the true organism, yet this difficulty is liable to arise when cultures are made. In such a condition, where pseudodiphtheria bacilli are present only, the inoculation of animals appears to give the only positive evidence of diphtheria.

Pharyngomycosis Leptothricia.—The term pharyngomycosis leptothricia has been applied to the peculiar formation of horny-like, white tufts in and about the tonsils, on the walls of the pharynx, and on the base of the tongue. Recently these white patches and tufts have received special attention, and it has been proved that many of them are examples of keratosis; others, however, deserve the term mycosis pharyngea leptothricia. The first clear description of the latter condition appeared from the pen of B. Fränkel.* Siebenmann contributed an exhaustive and admirable paper on this subject in 1895.

Collection.—Remove a small portion of one of these patches by means of a small forceps, smear thinly upon a slide, fix by heat, and stain with a solution of iodopotassic iodid. In a number of instances the Leptothrix buccalis will be found to be stained a bluish-red color (Fig. 193). This organism may also be recovered from the tartar of the teeth and from the mucous membrane surrounding the white patches.

Keratosis.—Recently C. W. Richardson † has described in detail these patches of so-called mycotic disease of the pharynx. In his paper Richardson states that in a large percentage of cases the condition is a true keratosis, and that the occurrence of the leptothrix in the patches is either accidental or physiologic, but that the organism bears no causal relation to the condition. Although there are probably various types of the Leptothrix buccalis, and while the earlier writers regarded this organism as the sole cause of the condition, more recent investigators (A. Brown Kelly) regard the disease as possibly due to bacteria other than the leptothrix. According to this view the bacteria induce certain changes in the lacunar epithelium (epithelial buds), which eventually result in the production of the white masses and horny quills. The leptothrix filaments are found in large numbers in specimens obtained from the tonsils, tongue, and nasopharynx. Organisms have been isolated from all these situations which have been practically identical with those recovered from the white patches previously described.

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* "Berlin Med. Soc.," 1873.

† "Amer. Jour. Med. Sci.," Oct., 1902.

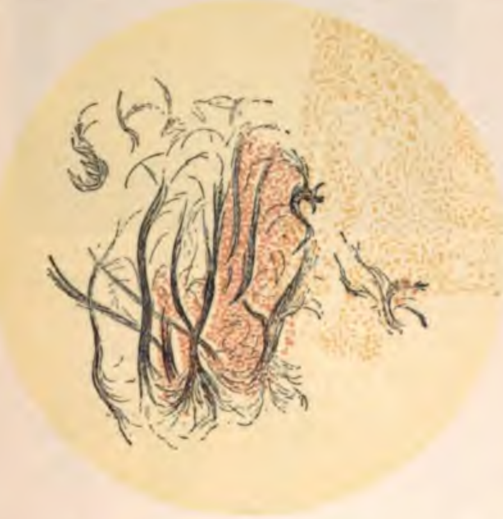


Fig. 193.—Leptothrix buccalis from the sputum of a phthisical patient. Fresh specimen treated with lactic acid and Lugol's solution (× about 300).

Cultures from the Throat.—Löffler's blood-serum will be found most serviceable for the cultivation of all bacteria common to the buccal secretion (see outfit, Fig. 194). Place the patient in a bright light, direct him to open his mouth well, or if a child see figures 195 and 196, and by means of a spoon or a tongue

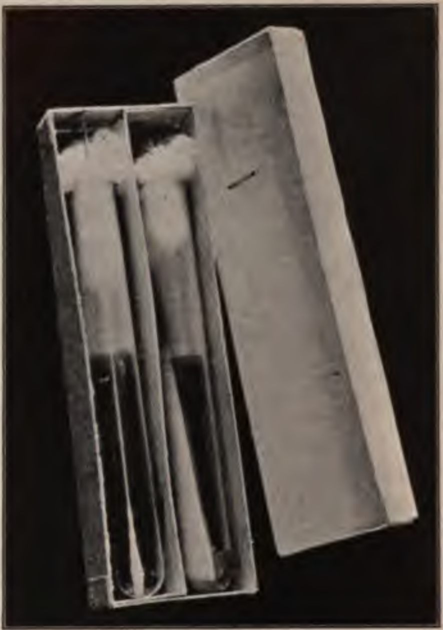


Fig. 194.—Health Department outfit for diphtheria diagnosis. A pasteboard box containing a swab-tube and a serum-tube, both with etched surface on which to write the name and address of patient, etc. (Gorham).

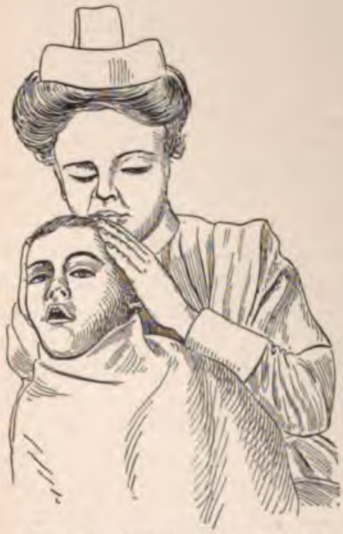


Fig. 195.—Method of holding a child when examining the throat: a blanket is wrapped to hold the arms by the side.

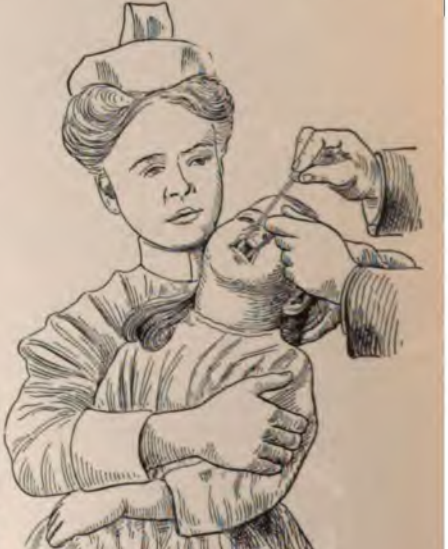
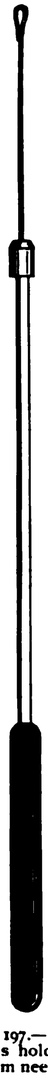


Fig. 196.—Making culture from throat. Left hand of nurse on child's occiput.

ressor obtain a clear view of the throat. Place a small amount terilized cotton upon a platinum needle (Fig. 197); draw the cotton over the false membrane of the tonsil, pharynx, or buccal mucosa, applying sufficient pressure to dislodge as much of the membrane as possible. Carry this cotton and its contained secretion into the culture-tube containing Löffler's blood-serum (Fig. 198), and smear the cotton over the entire surface of the medium. Cork tightly with the cotton stopper and place the tube in an incubator for twenty-four hours, when examine for colonies of the Klebs-Löffler bacillus and for other bacteria. In case of diphtheria the Klebs-Löffler bacillus may be present in a pure culture, but it is the rule to find it in conjunction with other organisms. At times the streptococcus may be the predominant organism, and a common finding is but few Klebs-Löffler bacilli with many colonies of staphylococci and of a large diplococcus. The streptothrix is also commonly encountered, and may appear in pure cultures in cases of pharyngomycosis.



ig. 197.—Rosen-
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num needle.

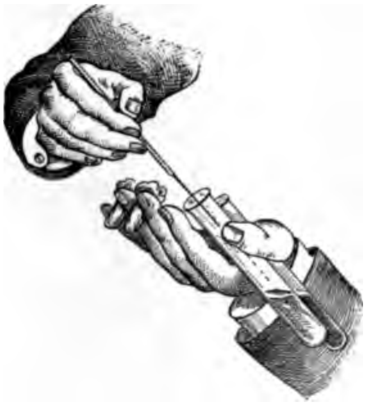

Fig. 198.—Method of holding tubes during inoculation.

Staining.—Remove one of the colonies that y have developed at the end of twenty-four hours by means a platinum needle, and spread it thinly upon a slide, fix by t, and stain for from three to five minutes with Löffler's

methylene-blue solution. Diphtheria bacilli are seen t
in a rather characteristic manner, their extremities staining
(Plate 31).

Other pathogenic bacteria are also stained by this s
Tuberculous conditions of the throat are to be recogni
staining the secretion for the tubercle bacillus (page 438
culture studies of this organism from the buccal secreti
impracticable.

CHAPTER VII.

THE NASAL SECRETION.

Naked-eye Appearance.—In health the nasal secretion is comparatively scanty, but in certain diseased conditions, either acute or chronic, it may be profuse. Normally the secretion is a clear, tenacious, odorless liquid, having a saline taste, and presenting a slight beaded froth. It is composed principally of mucus. Microscopically this secretion is found to contain epithelial cells in abundance, leukocytes, and fungi. Some epithelial cells show cilia, while others exhibit the various degenerative processes.

Reaction.—The reaction of the nasal secretion is alkaline.

Rhodan.—Rhodan may be present in the nasal and in the buccal secretions. It is detected by placing a drop of the secretion on filter-paper that has been previously colored yellow by a solution of ferric chlorid in hydrochloric acid. If rhodan is present, the yellow paper will turn red. Keller * discovered that rhodan is present in the nasal secretions of children two and three months old, but not in their saliva.

Pathologic Secretion.—In acute inflammatory processes of the nasal mucosa the secretion at first is diminished in amount, but following this stage it becomes profuse. In the latter condition numerous epithelial cells, together with a number of bacteria, may be found. Pus is present only when ulceration ensues, and its quantity bears a direct relation to the degree of ulceration. Chronic suppurative processes may affect both the nasal mucosa and the accessory air-spaces, such as the antrum, the ethmoidal air-cells, etc.

Hay-fever.—During the course of hay-fever the nasal secretion will be found to vary greatly at different hours of the day, depending upon the occurrence of the characteristic paroxysms of the disease. Early in one of these paroxysms the nasal secretion is lessened, but after the attack of sneezing the secretion becomes profuse. Microscopically the nasal secretion in this disease resembles that of an acute inflammation and often contains red blood-cells.

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* "Münchener med. Wochenschr.," Nov. 13, 1900.

Tuberculosis.—Tubercle bacilli may be found in the nasal secretion whenever there is a tuberculous ulceration of the nasopharynx. In my experience it has been rather common to find this organism present in the discharge from the nose in cases of large pulmonary cavities and in cases of tuberculous laryngitis. The finding of this bacillus in the nasal secretion is, therefore, of limited clinical value.

Meningitis.—The cerebrospinal fluid may escape into the nasal cavity as a result of fracture of the base of the skull. Cerebrospinal fluid has also been detected in the nasal secretion in cases of cerebral tumor and of caries of the bones of the skull.

Detection.—The presence of this fluid may be detected in the nasal secretion by the fact that it is practically free from albumin and that it contains a questionable body which is capable of reducing Fehling's solution.

The Diplococcus intracellularis of Weichselbaum (Fig. 213) may be found in the nasal secretion early in the course of epidemic cerebrospinal meningitis. This organism is said to have been found in the nasal secretion in healthy persons, but my experience does not confirm this statement. Out of twelve fatal cases of epidemic cerebrospinal meningitis I was able to detect the Diplococcus intracellularis in the nasal secretion in eight, both in cover-glass preparations and by culture. In staining for this organism a portion of the mucus or mucopus is smeared in a thin layer upon a slide, fixed by heat, stained for fifteen seconds with a solution of anilin-gentian-violet, and washed in water. Apply Gram's solution for one minute and alcohol until all the violet color disappears; wash again in water, and stain for two minutes with a saturated alcoholic solution of Bismarck-brown. The Diplococcus intracellularis will be found to be stained brown; the nuclei of the pus-cells and leukocytes, a mahogany-red; while the protoplasm of the cells will be stained a light brown. The meningococcus is often found within the bodies of the pus-cells,—a feature also characteristic of the gonococcus,—but, on the other hand, many extracellular diplococci are also present (see *Meningitis*). Without such an examination it is unwise to venture a positive diagnosis.

Glanders.—During the course of glanders the nasal secretion is most likely to contain the Bacillus mallei in great numbers.

Ozena.—In ozena the nasal secretion is said to contain many large diplococci, and, according to Lowenberg, this finding is fairly constant. Schlafrig* recovered a pure culture of the

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* "Wien. klin. Wochenschr.," Oct. 17, 1901.

Micrococcus tetragenus in this disease. Fungi (Oïdium albicans), cocci, and bacilli may also be found.

Klemperer and Sheier, in a rather exhaustive monograph upon the bacteriology of ozena and rhinoscleroma, conclude that the so-called bacillus of ozena resembles closely the bacillus of Friedländer (Fig. 188), and these authors believe that the latter organism becomes plentiful in the nasal secretions during the course of ozena.* In a few instances the tubercle bacillus has been recovered from the discharge in cases of ozena.

Leprosy.—Whenever leprosy involves the nasal cavity or the adjacent tissues, lepra bacilli may be found in the nasal secretion. They may be detected by the method described on page 112.

Plague.—In the pneumonic form of bubonic plague the nasal secretion may be found to contain the plague bacillus, but this finding is of but little importance, since the organism is readily recovered from other situations (see *Plague*, page 114). In plague pneumonia the sputum contains plague bacilli (Plate 31).

Pneumonia.—It is not uncommon to find the pneumococcus in the nasal secretion during the course of acute lobar pneumonia, and I have repeatedly isolated it from the nasal cavities of healthy persons. In one instance I recovered the bacillus of Friedländer from the nasal secretion in a case of lobar pneumonia.

Influenza.—The influenza bacillus may be detected in the nasal secretion early in the course of influenza. The bacilli appear as very slender rods which stain readily with carbolfuchsin (see *Sputum*).

Typhoid Fever.—During the course of typhoid fever many microörganisms may be obtained from the nasal secretion, and while the typhoid bacillus is at times present, the finding is of no clinical importance. Streptococci, diplococci, and bacilli are also present. The nasal secretion is likely to contain many red blood-cells, but the other evidences of inflammation are wanting.

Crystals.—Charcot-Leyden crystals (Fig. 189) are often to be seen in the nasal secretion, as are also crystals resembling the triple phosphates in appearance.

Fungi.—Molds are occasionally recovered from the nasal secretion, and a few records have appeared in the literature in which the thrush fungus and the aspergillus have been detected.

Entozoa.—Ascarides and oxyurides are reported as having been found in the nasal secretion. The larvæ of certain insects have also been found in the nasal cavity, but have rarely been detected in the discharge.

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* "Zeit. f. klin. Med.," vol. xlv, pts. 1, 2.

CHAPTER VIII.

DISCHARGES FROM THE EAR AND EYE.

THE EAR.

DURING the past few years the discharge from the middle ear has been studied in connection with both acute and chronic inflammations of the middle ear, as well as with catarrhal processes of the external auditory canal. Among the organisms commonly encountered in this situation should be mentioned the pneumococcus, the Streptococcus pyogenes, both of which are commonly associated with acute otitis media, the Staphylococcus pyogenes albus, the Staphylococcus pyogenes aureus, the Bacillus pyocyaneus, the bacillus of Friedländer, and the Bacillus coli communis. During the course of epidemic cerebrospinal meningitis the Diplococcus intracellularis may be found in the auditory discharge, as was observed in a case studied at the Philadelphia Hospital. The typhoid bacillus has been recovered from the auditory discharge in cases in which otitis media was found to complicate typhoid fever. When diphtheria involves the middle ear, the diphtheria bacillus may be detected in the auditory exudate. The tubercle bacillus has also been found in cases in which there is tuberculous involvement of the ear or the adjacent structures. During the course of influenza the bacillus of influenza may be found within the middle ear or in the pus discharge from the canal. There appears to be some question as to the exciting cause of acute otitis media, and it may safely be said that the laboratory worker is seldom called too early to recover the pneumococcus and the streptococcus, but, according to the researches of Lermoyez and Helme,* otitis media is of monobacillary origin, and the pneumococcus or the streptococcus is seldom to be found with other micro-organisms. At any rate, the disease has not progressed far when the pus becomes contaminated with staphylococci, so that it is not uncommon to find the latter organism only immediately after paracentesis. The fact that disease of the ear is commonly secondary to disease of the nasopharynx renders it reasonable to suppose, at least, that the same organisms are con-

* "Annal. d. Mal. de l'Oreille," 1895.

cerned in the production of the two conditions, and that they may be recovered from the pharyngeal and nasal mucosæ as well as from the middle ear—*e. g.*, during the course of scarlet fever streptococci and diplococci may be found in the throat, as well as in the discharge from the ear.

PARASITIC INFLAMMATION OF THE EXTERNAL AUDITORY CANAL.

(Otomycosis; Myringomycosis aspergillina.)

Mycosis of the external auditory canal is commonly of parasitic origin, and, in a great many instances, this condition is excited by the development of a rather common fungus, the Aspergillus niger, although other species of this organism have also been found—Aspergillus flavus and fumigatus. In addition to these organisms the Vesticillium graphii, Aspergillus fumigatus with grass-green conidia, Aspergillus nidulans, Ascophora elegans, Mucor corymbifer, Eurotium malignum, Mucor septatus, and the Penicillium minimum have been recovered from the external auditory canal.

Detection.—Remove a small portion of the mycotic débris and spread it thinly on a slide; add a small drop of water and apply a cover-glass. When studied under a one-fourth or one-sixth objective, many small, tape-like structures (hyphæ) may be seen (Plate 21), many of which support a spheric, granular body at one extremity (sporangium). Surrounding the sporangium several small spheric bodies (spores) are usually seen, which have been detached during manipulation (Plate 21). The various forms of aspergillus may be known partly from the color of the growth they produce, which depends somewhat upon their conidia. The Aspergillus niger produces a blackish or dark-brown growth; A. flavus, a yellowish or green growth, which is also produced by the A. glaucus; while a dull-gray or grayish-brown growth is characteristic of the A. fumigatus. I have found that the culture-medium and the degree and character of the light influence the color of the growth of the aspergillus materially—*e. g.*, when grown under a red, green, or blue glass the characteristic color of the fungus was greatly changed.

Sterigmatocystis Candida.—S. E. Cook* reports a case in which this fungus was found in the external auditory canal. The author claims his case to be the first in which this parasite (Fig. 199) was proved to be the cause of auditory mycosis.

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* "Amer. Med.," Dec. 5, 1903.

LARVÆ IN THE AUDITORY CANAL.

Living larvæ of dipterous insects may be detected in the human external auditory canal. W. E. Baxter,[*] in addition to reporting a case, makes reference to a number of instances where such larvæ have been recovered from this situation. As early as 1871 C. J. Blake contributed an interesting monograph upon this subject,[†] and several additional reports of the recovery of living larvæ from the external auditory canal have also crept into the literature, among which it may be well to make special mention of a report by C. W. Richardson.[‡] In Richardson's case there was a blood-stained purulent discharge from both ears. One living larva of a fly was removed from the right external auditory canal; and from the left canal two living worms were removed. The age of the child from whom these larvæ were removed was four months.

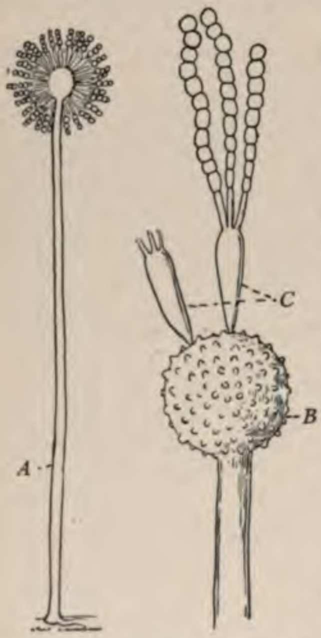

Fig. 199.—Sterigmatocystis candida: *A*, Fertile hypha; *B*, terminal vesicle; *C*, sterigmata, one showing chains of conidia.

THE EYE.

In health the secretion of the conjunctiva concerns us but little, although when studied microscopically it will be found to contain a few epithelial cells and small corpuscles. During the course of inflammatory processes of the conjunctiva, on the other hand, the secretion may be greatly increased and may at times become purulent.

Again, it may remain comparatively clear, although it contains a profusion of bacteria.

Gonorrheal Conjunctivitis.—Early during the course of gonorrheal conjunctivitis a variable quantity of pus may be seen exuding from between the folds of the swollen conjunctiva.

[*] "Arch. of Otol.," vol. xx, No. 1.

[†] "Arch. Path. and Otol.," vol. ii, pp. 37–44, No. 2

[‡] "Arch. of Otol.," 1895, vol. xxiv, Nos. 3, 4.

Collect a small drop of this discharge upon a platinum needle or upon a tooth-pick and smear it thinly over a slide; fix the specimen by passing it through the flame of a Bunsen burner, and then stain for the gonococcus (see *Gonorrhea*, page 494). Early during the course of the disease, when the pus is profuse, it has been my experience to find a pure culture of the gonococcus; but after the application of judicious treatment, especially silver-nitrate solution, the gonococci lessen in number and at times other bacteria (streptococci and staphylococci) appear in the discharge. In fact it is not uncommon, late in the disease, to find a rather large quantity of purulent discharge which, upon microscopic examination, is found free from bacteria.

Gonorrheal conjunctivitis should probably be considered as a variety of ophthalmia neonatorum, although not all cases of this condition are found to be due to infection with the gonococcus. Indeed, I have examined the pus from the conjunctiva of a number of children in which it was impossible to detect gonococci.

When judicious treatment has been instituted early, it may be difficult to detect gonococci in the discharge after the fourth or the fifth day of the disease; yet the exact stage at which gonococci disappear from the pus is apparently influenced by the individual resistance of the patient, the type of infection, and the treatment.

The characteristic feature of the gonococcus is that it is situated within the pus-cells and the epithelium. Early in the course of ophthalmia many extracellular cocci are seen, and, in fact, these may appear in rather dense aggregations, resembling the staphylococcus (Plate 34). The appearance of the streptococcus or of the staphylococcus in the pus may be regarded as a precursor of the disappearance of the gonococcus.

Keratitis.—In cases of keratitis various micro-organisms may be detected in the discharge from the conjunctivæ, and at times the same organisms are recovered from the nasal passages. The following organisms have been found in this connection: Staphylococci, streptococci, Pfeiffer's capsulated bacillus, pneumococci, Bacillus pyogenes fœtidus, Bacillus coli communis, the Bacillus pyocyaneus, diplobacillus, ozena bacillus, influenza bacillus, and tubercle bacillus. Certain fungi are also to be found, among which the Aspergillus fumigatus represents the one of pathologic importance. Uhthoff recovered lepra bacilli from the conjunctiva in a case of leprosy, and Posey found the same organism in a case of leprosy with chronic conjunctivitis.* The micro-organisms present in a given case depend upon the character of the ulcer in that case; a sloughing ulcer may result from one or many bacteria. In a number of cases, many of which were studied at the Philadelphia Hospital through the courtesy of Dr. de Schweinitz, I found that mixed infection was common. Certain of the ulcers produced a discharge which contained the pneumococcus only (Fig. 200); other discharges contained pneumococci and staphylococci; while a third series displayed streptococci in addition. At the suggestion of Dr. de Schweinitz cultures and smears were made from the margins and also from the centers of these ulcers.

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* "Jour. Amer. Med. Ass.," Oct. 3, 1903.

At times the marginal culture was found to contain only diplococci, staphylococci, or streptococci, while near the center of the ulcer more than one, and at times all, of these organisms existed. Occasionally the reverse condition was found to exist. In none of the cases studied was I able to detect the pseudodiphtheria bacillus.

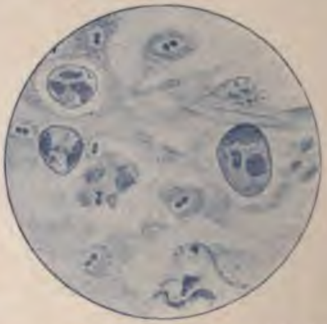

Fig. 200.—Diplococcus pneumoniæ from ulcer of cornea (obj. B. and L. one-twelfth oil-immersion) (study through courtesy of Dr. C. A. Oliver).

Epithelial cells and a variable amount of tissue débris are to be found in the scrapings from corneal ulcers.

Parasitic Keratitis.—In a case in which keratitis was complicated by hypopyon a keratotomy released a foreign body which was determined to be Hypoderma bovis, larva of the warble fly.*

Trematodes of the Eye.—Thus far there appear to be authentic records of but two instances where trematodes have invaded the human eye. These parasites have been referred to under a variety of names, and from the number of references to this subject one might at first glance suppose that trematodes were commonly found in this situation. Stiles has discussed at length the limited importance of this parasite in its relation to man, and has considered the trematodes invading the eye (Plate 32) under

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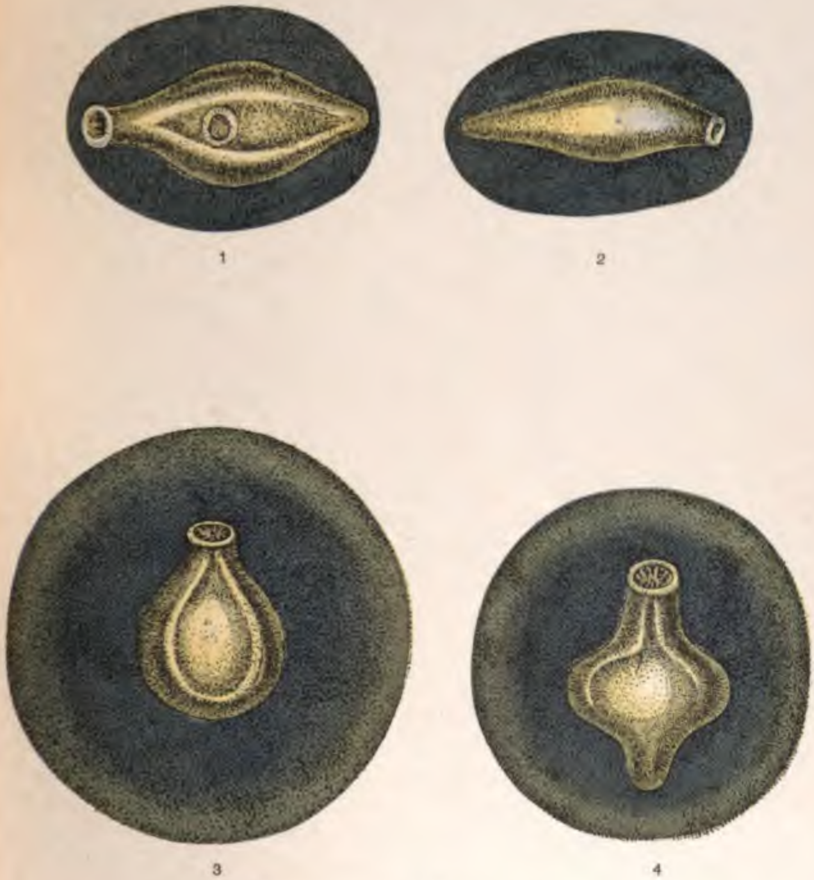
* "Hygeia," Sept., 1901.



AGAMODISTOMUM OPHTHALMOBIUM.

1, Ventral view; 2, dorsal view; 3 and 4, two other views in different stages of contraction (after von Ammon).

the terms Monostomulum lentis and Agamodistomum ophthalmobium.*

Keratomycosis.—According to Ball,† less than a dozen cases of this affection are described in the literature. The mycotic area appears as small black bodies within the substances of the cornea. Upon removal from the cornea the mass may be crushed upon a slide, a drop of water added to it, and the specimen brought into focus under a one-fourth or a one-sixth objective. It will be found to consist for the most part of mycelial threads, a few of which show sporulation (sporangia), spores, and a variable number of pus-corpuscles (Fig. 201). This fungus, when present in the black bodies, is suggestive of Aspergillus niger, although I am not aware that this organism is commonly the cause of pathologic conditions. On the other hand, the Aspergillus fumigatus, which usually produces a brownish or yellowish-green growth, is highly pathologic for animals. Osterroht ‡ reports a similar case.

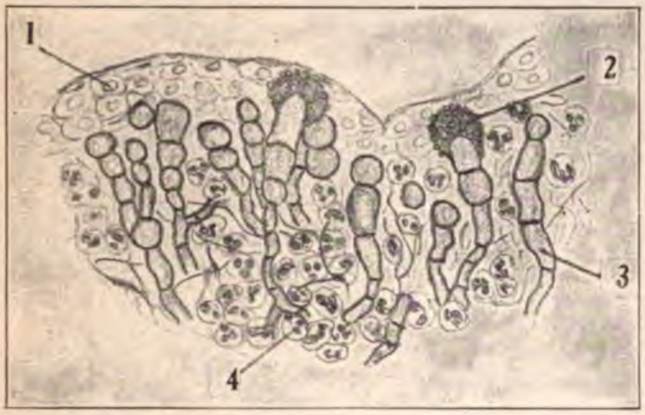


Fig. 201.—Microscopic appearance of a section of the growth in aspergillar keratitis: 1, Epithelial surface of cornea; 2, sporangium; 3, mycelium; 4, leukocytes or pus-cells (Ball).

In so far as I have been able to learn from the literature, the organism found in aspergillous keratitis is probably one of these fungi, though there is no record of cultural studies of this parasite (see *Culture of the Aspergillus*, page 469). In Ball's cases the black masses removed from the cornea were sectioned.

Keratoconjunctivitis.—In keratoconjunctivitis it is fair to presume, at least, that the same etiologic factors that have been described under the head of keratitis are active. When the disease is of a carcinomatous nature, a portion of the growth may be removed for pathologic study; but scrapings from the conjunctiva have never given sufficient evidence to enable me to make a positive diagnosis.

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* U. S. Department of Agriculture, Bureau of Animal Industry, Bulletin No. 35.

† "Amer. Med.," July 6, 1901, p. 31. ‡ "Berliner klin. Wochen.," Feb. 13, 1905.

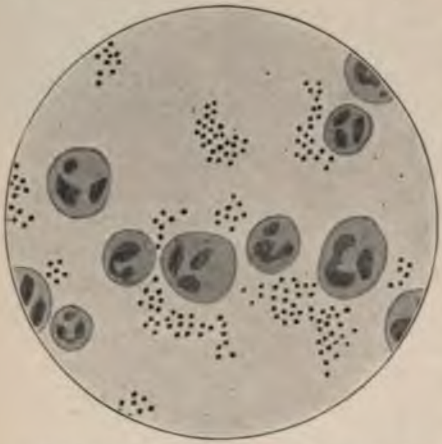

Fig. 202.—Staphylococcus pyogenes aureus in exudate from corneal abscess. Observed at Philadelphia Hospital (obj. Queen one-twelfth oil-immersion).

Abscess of the Cornea.—In four cases of abscess of the cornea Staphylococcus aureus was present in two (Fig. 202); the Staphylococcus pyogenes, Staphylococcus pyogenes albus, and the Streptococcus pyogenes were found in the third, and the pneumococcus and a staphylococcus of a questionable nature were present in the fourth. In these cases the pus was released by the surgeon and collected as it exuded from the cornea. In this connection tuberculosis of the cornea and suppurative keratitis should be mentioned, as they are also of bacterial origin.

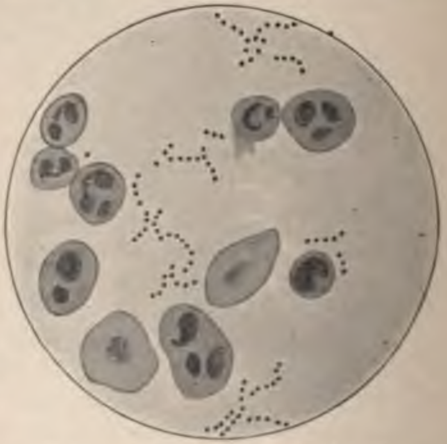

Fig. 203.—Streptococcus pyogenes from the exudate during acute membranous conjunctivitis. Observed at Philadelphia Hospital (obj. Queen one-twelfth oil-immersion).

Diphtheric Conjunctivitis.—The exudate from a case of diphtheric conjunctivitis may show broken-down epithelial cells, leukocytes, débris, and a growth of streptococci (Fig. 203). A false membrane may result, according to some authors, from the development of the leptothrix (Fig. 193). This organism may accumulate to form a rather dense mass in the conjunctival tissue.

This mass, subsequently becoming calcified, forms the so-called "tear stone." In instances in which the Klebs-Löffler bacillus is found in the scrapings and in the cultures from the conjunctiva the condition should be regarded of diphtheric nature.

Caution.—A fact ever to be borne in mind is that the diphtheria bacillus is not essential to the production of a false membrane.

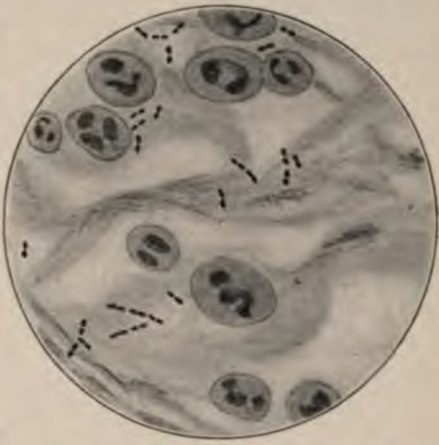

Fig. 204.—Morax-Axenfeld diplobacillus from conjunctival exudate during course of subacute conjunctivitis. Patient observed at Philadelphia Hospital (obj. B. and L. one-twelfth oil-immersion).

Trachoma.—In trachoma the granular lids have not been proved to be due to the diplococcus of Sattler or of Michel; "neither has the trachomacoccus been identified; indeed, some observers (Mutermilch) deny its existence" (de Schweinitz). Noiszewsky has described a fungus which, in many respects, resembles the Microsporon furfur, and has found this organism pathologic for rabbits. Pfeiffer has described a protozoön in connection with trachoma, but the true organism upon which this disease depends is not definitely known.

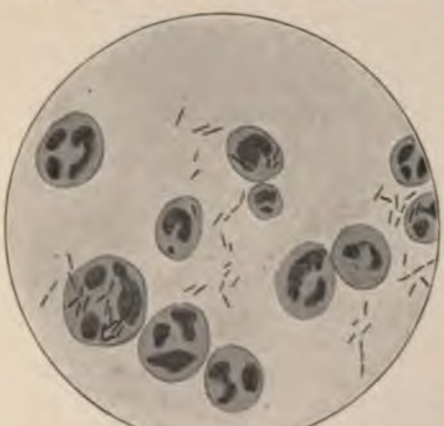

Fig. 205.—Koch-Weeks' bacillus from conjunctival exudate at third day of epidemic conjunctivitis (obj. Spencer one-twelfth oil-immersion).

Acute Infectious Conjunctivitis. — Koch has described a specific bacillus which he found commonly associated with the conjunctival secretion in acute infectious conjunctivitis. Morax has also studied this organism in Europe, as well as a diplococcus (Fig. 204). It has been extensively studied in America by Dr. Weeks, of New York. According to Koch's description, this bacillus resembles the organism of mouse septicemia in certain respects. It measures from one to two μ in length by 0.25 μ in breadth, and stains readily with the ordinary anilin dyes. The Koch-Weeks' bacillus (Fig. 205) grows well upon the addition of hemoglobin to the surface of the culture-medium. The bacillus of influenza (Fig. 187) has been suggested as being identical with this organism. At times this may be the only organism present in the conjunctival secretion, although it is commonly associated with a bacillus resembling that of diphtheria (xerosis bacillus). It has been claimed by Rimovitch * that the bacillus of acute infectious conjunctivitis is identical with the influenza bacillus.

Parasitic Cysts.—The small hard sacs of the eyelids have been studied by Hunsche,† who found that omitting young children, in whom this disease is not usual, 92.5 per cent. of cases are due to the Demodex folliculorum, a minute parasite known to infest the sebaceous follicles of man and of certain animals. This parasite does not, as a rule, excite appreciable inflammation.

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* "Russki Archiv Pathologii," etc., vol. xii, No. 2.

† "Münchener med. Wochenschr.," Nov. 6, 1900.

CHAPTER IX.

SECRETION OF THE GENITAL ORGANS.

SEMEN.

THE ejaculated secretion of the male organs of generation is derived from a number of sources: the testicle, the prostate gland, the seminal vesicles, Cowper's glands, and the glands of Littre. When there is an inflammatory process of any or all of these glands or of the genital mucosa, the secretion may present features which may vary according to the pathologic condition present. Again, a disproportionate activity of one gland or set of glands often results in an abnormal appearance of the secretion.

Method of Collection.—Direct the patient to prepare a vaselin bottle which is provided with a well-fitting cork. If the semen is to be collected at the time of sexual intercourse, the penis should be withdrawn just before ejaculation and the semen allowed to escape into the bottle. The bottle should be corked tightly and kept warm (37° C.—98.6° F.) until a careful study has been completed. Semen collected after masturbation or after an involuntary emission is equally valuable for study. When it is necessary to convey the semen for a considerable distance I have found the following plan to serve well for keeping the spermatozoa active: Place a quantity of fresh semen in a bottle two inches long by one-half inch in diameter and cork tightly. Attach a string of sufficient length to the neck of this bottle, so that when suspended from a button on the trousers, the bottle will hang against the skin of the inguinal region. In this way the semen is kept warm and may be carried for hours without the spermatozoa losing their motility.

Characteristics.—The seminal fluid when first ejaculated is a thick, white, bluish-white, or yellowish, opaque, tenacious, semi-gelatinous fluid of an alkaline reaction. Upon standing exposed to the air it soon becomes opaque and gives off a characteristic sweetish odor. Fresh semen will be found to sink when placed in water, collecting as a gelatinous mass at the bottom of the vessel. When shaken with water, the cellular elements are found to be distorted.

Chemistry.—According to the analysis of Vauquelin a Kollicker, mammalian semen is composed of the following:

	Man.	Horse.	Bull.
Water	90.0	81.9	82.1
Albuminous material	..	..	15.3
Extractives	6.0	16.45	..
Ethereal extract	..	..	2.2
Mineral substances	4.0	1.61	2.6

The mineral constituent ordinarily found in semen is calcium phosphate.

Microscopic Study.—Place a drop of the semen upon a slide and permit it to remain exposed to the air for a short time; then apply a cover-glass and examine microscopically for the so-called Charcot-Leyden crystals (Fig. 189). Other needle-like crystals, which are probably phosphates, may also be found. Warm a slide to body-temperature and place a drop of semen upon its center. Repeat the warming process from time to time, carrying the slide to the microscope between each heating and bringing it into focus under a one-sixth or a one-eighth objective. When thus examined, the semen will be seen to contain many actively motile bodies which, upon careful focusing, are best described by the accompanying illustrations (Plate 33).

Spermatozoön.—The male cell, or spermatozoön, is of minute size, and in its locomotive energy and vitality resembles a flagellate monad. Anatomically the spermatozoön is a true cell; it is composed of a head, a middle piece, and a tail or flagellum. The head of the spermatozoön corresponds to the nucleus of a somatic cell, and is, therefore, composed principally of chromatin. The middle piece contains the centrosome. The tail, which is actively motile in the living cell, contains a central axial filament. This axial filament starts from the centrosome in the middle piece and runs through the entire length of the tail. Human spermatozoa will be found to resemble the spermatozoa of certain other mammals* in many respects (see Plate 33, in which a comparative study of the spermatozoa of man, domestic animals, and rodents is illustrated).

In studying the spermatozoa of the mastiff I noticed the striking resemblance they bore to those of man. I also found that if the spermatic fluid were allowed to stand for a time, even staining did not furnish sufficient evidence to enable me to distinguish beyond question the spermatozoa of man from those of the dog. Only when careful measurements were employed was the differential diagnosis possible. A fact ever to be borne in mind is that these

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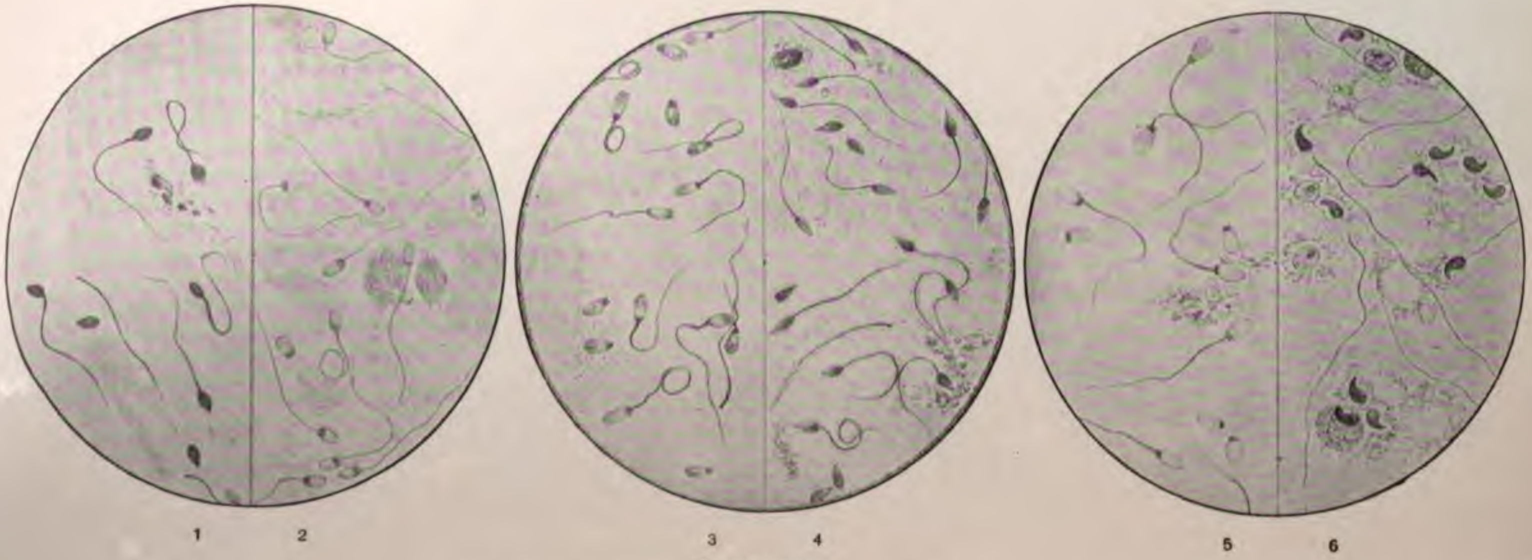
* Boston, "Jour. App. Mic.," vol. iv, No. 7, p. 1360, Rochester, N. Y.

PLATE 33.

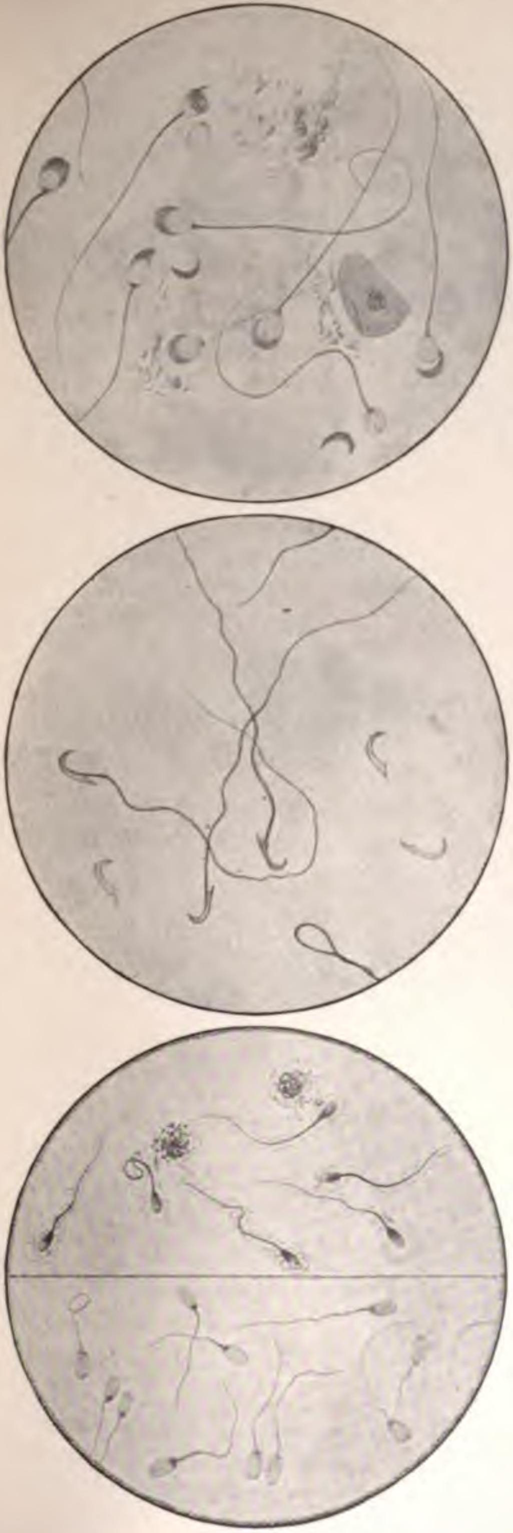


SPERMATOZOA.

1, Man; 2, dog (mastiff); 3, rabbit; 4, horse; 5, bull; 6, mouse.

(*Continued on next page.*)

PLATE 33—(Continued).



SPERMATOZOA.

7, Sheep; 8, cat; 9, white rat; 10, guinea-pig.

measurements may vary slightly in different persons and in animals, and even in spermatozoa from the same specimen of semen. In this connection I am prompted to offer the following table of measurements, obtained by personal observations:

SPERMATOZOA OF MAMMALS.

MAN.

Total length	0.051–0.058 mm.	(0.002 –0.0022 in.)
Length, head	0.004–0.006 mm.	(0.0001–0.0002 in.)
Width, head	0.003–0.004 mm.	(0.0001–0.0001 in.)
Length, tail	0.041–0.053 mm.	(0.0016–0.002 in.)

DOG.

Total length	0.067–0.074 mm.	(0.0026–0.0028 in.)
Length, head	0.004–0.008 mm.	(0.0002–0.0003 in.)
Width, head	0.003–0.002 mm.	(0.0001–0.0001 in.)
Length, tail	0.059–0.067 mm.	(0.0023–0.0026 in.)

RABBIT.

Total length	0.051–0.066 mm.	(0.002 –0.0025 in.)
Length, head	0.006–0.009 mm.	(0.0002–0.0003 in.)
Width, head	0.003–0.004 mm.	(0.0001–0.0001 in.)
Length, tail	0.045–0.058 mm.	(0.0017–0.0022 in.)

HORSE.

Total length	0.064–0.067 mm.	(0.0025–0.0026 in.)
Length, head	0.006–0.008 mm.	(0.0002–0.0003 in.)
Width, head	0.003–0.004 mm.	(0.0001–0.0001 in.)
Length, tail	0.054–0.06 mm.	(0.0021–0.0022 in.)

BULL.

Total length	0.087–0.093 mm.	(0.0033–0.0036 in.)
Length, head	0.009–0.009 mm.	(0.0003–0.0003 in.)
Width, head	0.006–0.006 mm.	(0.0002–0.0002 in.)
Length, tail	0.077–0.083 mm.	(0.003 –0.0032 in.)

MOUSE.

Total length	0.12 –0.158 mm.	(0.0046–0.0061 in.)
Length, head	0.008–0.009 mm.	(0.0003–0.0003 in.)
Width, head	0.003–0.004 mm.	(0.0001–0.0001 in.)
Length, tail	0.112–0.138 mm.	(0.0043–0.0057 in.)

SHEEP.

Total length	0.083 mm.	(0.0032 in.)
Length, head	0.009 mm.	(0.0003 in.)
Width, head	0.006 mm.	(0.0002 in.)
Length, tail	0.074 mm.	(0.0028 in.)

CAT.

Total length	0.058–0.074 mm.	(0.0022–0.0028 in.)
Length, head	0.004–0.007 mm.	(0.001 –0.0002 in.)
Width, head	0.003–0.003 mm.	(0.0001–0.0001 in.)
Length, tail	0.053–0.066 mm.	(0.002 –0.0025 in.).

White Rat.

Total length	0.225–0.238 mm. (0.0087–0.0092 in.)
Length, head	0.012–0.016 mm. (0.0004–0.0006 in.)
Length, tail	0.209–0.222 mm. (0.0081–0.0086 in.)

Guinea-pig.

Total length	0.113–0.138 mm. (0.0053–0.0057 in.)
Length, head	0.006–0.012 mm. (0.0006–0.0004 in.)
Width, head	0.007–0.011 mm. (0.0004–0.0004 in.)
Length, tail	0.125–0.132 mm. (0.0048–0.0051 in.).

Semen also contains epithelial cells, hyaline bodies, and a few granular bodies which are not unlike starch-granules in appearance. Leukocytes are always present, and a few red blood-cells are usually demonstrable.

Stain.—Spermatozoa stain readily with the ordinary anilin dyes.

PATHOLOGY OF THE SEMEN.

In this country a careful study of the semen is sadly neglected, and in a case of a sterile marriage the fault is usually ascribed to the female. According to Kehrer, however, spermatozoa are absent from the semen in 40 per cent. of the cases of sterile marriages (azoöspermism).

My own experience in a rather large number of cases does not accord with Kehrer's findings, although I have found a number of instances in which sterility was dependent upon some pathologic state of the semen. In many instances no spermatozoa were present (azoöspermism); in others many well-formed spermatozoa were found, but they were devoid of motility; and in a third group of cases the heads and tails of the spermatozoa were separated. While these three findings have been common in my experience, I have never been able to learn from the genito-urinary specialist upon what pathologic conditions these changes depend. Spermatozoa are absent from the semen during convalescence from acute febrile conditions (typhoid fever), and in such chronic conditions as valvular heart disease, severe anemia, tuberculosis, and other states in which the general vitality of the individual is greatly reduced. In a number of cadavers examined postmortem I was unable to recover spermatozoa from the vas deferens.

Bacteria.—The semen may contain the gonococcus, the Staphylococcus pyogenes, the streptococcus, and, rarely, the tubercle bacillus.

VAGINAL SECRETION.

In health the vaginal secretion is scanty in amount—merely sufficient to keep the mucous membrane lubricated. It is clear or at times opalescent, semiliquid, and is composed for the greater part of mucus and epithelial débris. It is acid in reaction.

MICROSCOPIC STUDY.

Microscopically a large amount of cellular débris will be found, entangled in the meshes of which are epithelial cells, mononuclear leukocytes (large), mucus-corpuscles, and bacteria.

The bacteria to be found in the vaginal secretion concern us but little, since a profusion of micro-organisms may be present without causing the patient any inconvenience. Döderlein regards his special bacillus as the only organism constantly present in the normal vaginal secretion. This organism possesses the faculty of inducing a marked acid fermentation of sugar. Döderlein's claims have not been substantiated by the works of other equally careful investigators (Krönig and Menge). These observers have found cocci and bacilli present in vaginal secretions which were apparently normal. Such organisms belong to a class of anaërobes known to be non-pathogenic. It will be found that a variety of organisms may be cultivated from the lower portion of the vagina, while an entirely different group of organisms are obtained from the upper portion of the canal and from the cervix of the uterus.

Collection.—Separate the labia well and lift a small amount of the secretion by means of a platinum needle. Transfer this to the center of a clean slide, and smear thinly over the surface of this slide by means of a second slide (see *Blood-smears*, page 35). A number of slides may be prepared in this way by the gynecologist or by the general practitioner. Place the specimen surface of slide No. 1 up, and upon it place two bits of match-sticks. Rest the second slide, specimen surface down, upon these sticks. In this way a number of slides may be piled together so as to prevent the specimens from touching. The slides should be wrapped in paper and sent to the laboratory for examination. When it is desired to transfer the vaginal secretion to the laboratory in liquid form, Dr. W. Easterly Ashton has employed the tubes used for the collection of blood (Fig. 44). A small syringe is attached to one end of the capillary glass tube by means of rubber tubing. The free end of the capillary glass tube is brought in contact with the vaginal secretion, which is then drawn into the tube by the suction of the syringe. Remove the rubber tubing from the capillary glass tube, and pass the ends of the latter through the flame of a Bunsen burner, applying sufficient heat to seal it. Place in a test-tube or any suitable receptacle and convey to the laboratory.

Fixing.—Fix all smears of vaginal secretions by heat, either by passing them directly through the flame or by heating at a low temperature (60° C.—140° F.) for twenty minutes or more.

NORMAL AND PATHOLOGIC SECRETION.

The normal vaginal secretion will be found to contain many cocci and bacilli, none of which, however, are pathogenic. In many normal secretions a few cocci may be found which in their morphologic and staining properties are practically identical with the gonococcus, yet these organisms have not the power to excite urethritis in the male. I have found this organism in normal vaginal secretions, but have never found it within the protoplasm of the pus-cells or epithelial cells in the manner characteristic of the gonococcus (Fig. 206). It is almost impossible to mistake this organism for the gonococcus, because the latter, in a case of acute gonorrhea before treatment has been instituted, always occurs in great numbers and pus-cells are often seen which contain from 12 to 50 cocci (Plate 34). In a normal secretion containing innocent cocci I have never found the pus-cells and the leukocytes invaded to this degree; so that while the cultivation of the gonococcus is often recommended, it is an

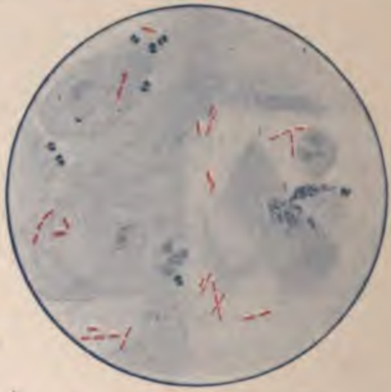


Fig. 206.—Vaginal secretion showing diplococci and smegma bacilli (obj. Spencer one-twelfth oil-immersion).

unnecessary proceeding, and, in addition, is most impracticable. It is impossible to inoculate animals with the gonococcus.

Gestation.—During the latter months of gestation it is common to find a profusion of bacteria in the vaginal secretion—bacilli, streptococci, staphylococci, and, occasionally, diplobacilli and streptobacilli are to be seen. In a series of experiments conducted at the Philadelphia Hospital a number of these organisms were isolated from the vaginal secretions of pregnant women and a series of animal inoculations made. I was unable, however, to produce pathologic lesions with these bacteria. Other observers have found that bacteria isolated from the vaginal secretions were pathologic for animals only when the vitality of the animal or of the part employed for inoculation was greatly reduced.

Bactericidal Properties.—Krönig was the first to demonstrate conclusively the bactericidal properties of the vaginal secretion by placing a number of micro-organisms within the healthy vagina and observing carefully at what length of time the vaginal secretion was capable of destroying them. Krönig found that it was impossible to recover the Bacillus pyocyaneus in from ten to thirty hours after its introduction into the vagina; the Streptococcus pyogenes disappeared after six hours; and the Staphylococcus pyogenes after from six to thirty-six hours.

Prompted by Krönig's investigations, I began a series of similar investigations at the Philadelphia Hospital,* and through them was able to confirm his work. More attention was directed to the effect of vaginal douches in the removal of these bacteria from the vaginal secretion. It was found that by douching the normal secretion—"the woman's safeguard against puerperal sepsis"—was removed and that the micro-organisms developed more rapidly. Furthermore, it was demonstrated that it is impossible to remove pathogenic bacteria from the vagina by means of antiseptics, but that they were always destroyed by the normal secretion when no douche was employed. A number of cases of gonorrhea in the female also received study, and it was found that cervical gonorrhea did not develop in the Philadelphia Hospital when the woman did not receive douches. On the other hand, cervical gonorrhea and pelvic trouble frequently followed the administration of douches.

Gonorrhea.—Urethral gonorrhea appears to be a fairly common condition, but vaginal gonorrhea must certainly be rare—thus far I have seen but a single case. Pus which contained gonococci has been obtained from the cervix uteri, but this is a much less common finding than we are led to believe. The gonococcus when present in the vaginal, urethral, or cervical secretion or in pus, is detected in the manner described for the study of this organism (page 494).

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* "N. Y. Med. Jour.," June 10, 1899.

Caution.—It has been my custom to teach that it is unsafe to make a diagnosis of gonorrhea in a female after puberty, since many other bacteria may be found which are not unlike gonococci; and in view of the great importance of such a diagnosis I should be inclined to rely upon the cultivation of the gonococcus, although in my own hands even this has not been satisfactory.

Ulceration of the Cervix.—In cases of ulceration of the cervix uteri the vaginal secretion usually contains a number of bacteria, and scrapings from the ulcer may show one or more pathogenic organisms to be present. These organisms in some cases are capable of producing a urethritis in a male who is exposed.

Carcinoma of the Cervix.—In cases in which there is a carcinomatous erosion of any portion of the vaginal canal masses of tissue which have sloughed may at times be recovered. When studied under the microscope, these masses are found to be quite characteristic of epithelial growths. Carcinoma, however, should be diagnosed after a section of the growth and not after examination of mere scrapings from the ulcerated surface.

Diphtheria and Noma.—Diphtheria of the vulva is certainly an uncommon condition, and yet it may be encountered. We should, therefore, be on guard for such a condition should it arise. The false membrane resembles that seen upon the other mucous surfaces, and when a portion of it is removed, spread thinly upon a slide, fixed by heat, and stained for five minutes with Löffler's methylene-blue solution, it will be found to contain a varying number of diphtheria bacilli.

Noma may at times involve the vulva. This condition is doubtless parasitic, and since the finding of the diphtheria bacillus by Sailer * in noma of the buccal cavity, the scrapings from such ulcers should be stained for this organism.

Animal Parasites.—The **Trichomonas vaginalis** is commonly seen in the vaginal secretion. It is apparently identical with the trichomonas recovered from the feces. This organism is not known to excite pathologic changes. It in no way resembles the spermatozoön (see page 478).

Oxyurides.—The vaginal discharge may be found to contain both the adult oxyuris and its ova, either of which are readily detected under a two-thirds or a one-sixth objective (see *Feces*, page 414).

* "Phila. Co. Med. Soc.," Nov., 1901, p. 301.

MENSTRUAL FLUID.

At the outset of menstruation the fluid is mucoid in character, but there is soon an admixture of red blood-cells until these cells so predominate that they determine the general characters of the fluid. With the decline of the flow the red cells decrease in number and the leukocytes make up the majority of the cellular elements found in the discharge. Large numbers of prismatic epithelial cells, showing areas of fatty degeneration and quantities of fatty débris, are also found at this stage of menstruation.

Abortion.—Chorionic villi are expelled with the blood-clots after an abortion. They are to be recognized by their club-shaped extremities and by their epithelial covering. It is also possible in certain instances to recover decidual cells which are of large size and may assume polygonal, spindle-shaped, or imperfectly rounded forms. The nuclei of these cells are especially conspicuous on account of their irregular contour and large size.

Lochia.—During the first twenty-four hours following delivery the lochia are red (lochia rubra) and present the characteristic odor of blood. The discharge is composed of mucus, red blood-cells, leukocytes, and vaginal epithelium. During the second and third days the numbers of red corpuscles lessen greatly, while the leukocytes are increased in number, and this increase continues day by day until the secretion becomes grayish or white in color.

From the tenth to the twelfth day the discharge becomes thicker, presents a mucoid appearance, is of a yellowish-white or milk-white color, and contains bacteria, among which streptococci and staphylococci are found. It is to be borne in mind that the bacteria found in the discharge at this time are, as a rule, slightly, if at all, pathogenic.

Membranes.—The retention of fetal membranes or the introduction of pathogenic bacteria into the birth-canal may cause a lessening or, at times, an absence of the lochia. This temporary decrease is followed by an excessive flow, which in some instances emits a decidedly fetid odor. Portions of fetal membranes or of placental tissue are often discharged with such lochia.

Vulvitis and Vaginitis.—In inflammations of the vulva and vagina there is a marked increase in the secretion, which is composed principally of leukocytes and epithelial cells. Variations in the character of these cells are observed, depending upon the portion of the mucous surface from which they are derived. Red blood-cells are also present when the inflammation is intense. Rarely, indeed, do we find large shreds of desquamated epithelium that have sloughed from the vaginal wall, and casts of the vagina have been observed. When ulceration occurs and even in some cases of non-ulcerative vaginitis, a variable amount of pus is present. Vaginovesical or vaginorectal fistulæ may permit the escape of the contents of the bladder or of the rectum into the vagina.

Pruritus Vulvæ.—Seligmann * states that in chronic pruritus vulvæ he has found a diplococcus constantly present; and that the number of diplococci found are in direct proportion to the degree of activity of the pruritus. Seligmann's diplococcus closely resembles the gonococcus, differing from this organism in that it stains by Gram's method and that it possesses decided cultural characteristics (see *Gonococcus*, page 494). The diplococcus present in pruritus vulvæ is destroyed by a 10 per cent. solution of guaiacol-vasogen.

Membranous Dysmenorrhea.—During seizures of dysmenorrhea the vaginal secretion is found to contain, in addition to blood, many shreds of desquamated mucous membrane, and I have seen a complete cast of the uterine cavity. Portions of this membrane, when teased, show blood-cells, epithelial cells, and débris. A far more satisfactory method of study is to treat this membrane by employing the ordinary methods for examining pathologic tissues.

Vaginal Blennorrhea.—The vaginal secretion is increased during sexual excitement, coitus, for some hours before the establishment of and during the menstrual period, and during the latter months of gestation, at which time a profuse creamy discharge may be physiologic.

Mycosis.—An increased vaginal secretion may result from a catarrhal process or it may be due in part to the existence of small mycotic areas situated near the orifice of the canal. Scrapings from these mycotic patches will be found to contain a profusion of bacteria and of fungi. Vaginal blennorrhea of gonorrheal origin has not been considered under this caption.

Fungi.—The thrush fungus is rarely found in the vaginal secretions.

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* "Deut. med. Woch.," Feb. 22, 1902.

CHAPTER X.

TRANSUDATES AND EXUDATES.

FLUID may be effused into the serous cavities of the body as the result of circulatory embarrassment (transudates) or of inflammatory processes (exudates). During health these serous cavities of the body contain quantities of fluid too small for analytic analysis. During pathologic processes, on the other hand, fluid may accumulate in any of the serous sacs, as well as in the areolar connective tissues immediately beneath the skin and between the muscles. When such conditions are the result of circulatory disturbances there is a deficiency in the elimination of water by the kidneys and the retained fluid collects in the serous sacs as transudates. As a result of inflammatory processes of the serous membrane there is a similar accumulation of fluid in the serous sacs, and such collections are known as exudates. These two conditions, while they are widely different in origin, often give rise to the same clinical manifestation, so that at times it is difficult to decide to which class a given fluid belongs. Again, from clinical evidence alone, ovarian cysts, hydatid cysts, and cystic kidneys are liable to be confused with ascites. In such a case chemic analysis of the fluid may be the only means of deciding the diagnosis.

TRANSUDATES.

Transudates are, as a rule, serous in character and of a straw color. At times, however, they may assume a sanguineous appearance from the admixture of blood, and in rare instances they are chylous. Transudates from the peritoneal cavity are usually of low specific gravity, ranging between 1.005 and 1.015, which is somewhat lower than that of the exudate. Pleural transudates the result of cardiac disease or cirrhosis of the liver usually are below 1.012 in specific gravity, although in some instances they may reach 1.015. Pleural effusions due to inflammatory processes alone usually have a specific gravity between 1.018 and 1.030. This range of specific gravity includes that of those effusions composed of pus. This variation in the specific gravity probably depends largely upon the albumins present in the fluid. Uric acid, a common constituent of both transudates and exudates, is most abundant where the specific gravity is high. This uric acid is derived from the nuclein of the corpuscles. The nitrogen and proteid materials likewise influence the specific gravity. Serum-albumin and serum-globulin may be detected in either an exudate or a transudate; in the former they may constitute from 3 to 5 per cent. of the fluid; while in the latter they usually constitute between 0.5 and 2.5 per cent. of the fluid. Ruess gives the following table:

Specific Gravity.	Per cent. of Albumin.	Specific Gravity.	Per cent. of Albumin.
1.008	0.2	1.019	4.3
1.009	0.6	1.020	4.7
1.010	1.0	1.021	5.1
1.011	1.3	1.022	5.5
1.012	1.7	1.023	5.8
1.013	2.1	1.024	6.2
1.014	2.5	1.025	6.6
1.015	2.8	1.026	7.0
1.016	3.2	1.027	7.3
1.017	3.6	1.028	7.7
1.018	4.0		

Transudates, when allowed to stand for a time, do not coagulate spontaneously, except when there is an admixture of blood—a feature which often serves to distinguish them from exudates. Exhaustive chemic analyses of transudates have been made by Hoppe-Seyler and Hammarsten.

MICROSCOPIC STUDY.

In the microscopic study of transudates, exudates, cystic fluid, and even mucopurulent exudates the first step necessary is that the fluid be collected in the proper manner; secondly, that its cellular elements be thrown down in the form of a sediment; and lastly, the further study of such sediments.

Collection.—Whenever practicable, fluid should be collected by aspirating the sacs in which it is contained, then permitting it to flow into a sterilized glass bottle or into test-tubes. In collecting fluid from the pleura it is always necessary to employ the special apparatus for this purpose; after which it is well to transfer some of the fluid to sterile test-tubes. Cystic and abdominal fluid may be allowed to flow directly into the test-tubes when collected after the method outlined under aspiration of the meninges or abdominal cavity, the same precautions being observed to prevent contamination of the fluid. Whenever it is possible, permit a portion of the fluid to flow directly into test-tubes containing culture-medium (see *Meningeal Fluid*, page 501).

Caution.—Far more clinical evidence is to be obtained through cultural and inoculation studies than from a mere microscopic study of either transudates or exudates. Fluid, whenever obtained from a serous cavity, should in addition to being placed in sterilized tubes be kept at a temperature of 37° C. (98.6° F.). Again, cultures should be made within a few hours after the fluid is removed from the body.

Sedimentation.—Fluids, either serous or other, in which there are but few cellular elements, are best studied by permitting them to stand for a few hours in order that a precipitate forms, and in case this be copious, further sedimenting is not required. The precipitate that has collected at the bottom of the fluid, when but slight, should be lifted by means of a pipet, and such sediments placed in a tube of the centrifuge. Transfer all the sediments from the receptacle to the tube, and then add sufficient of the liquid portion of the exudate to fill the tube; place in the machine and centrifugalize for from three to ten minutes, or until an appreciable amount of sediment has collected at the bottom of the tube.

Lift the sediment from the bottom of the tube into a small pipet, and place a small drop of the thickest of this upon the center of a clean slide. Smear this drop thinly over the slide by using a second slide (see *Method of Making Blood Smears*, page 35), and permit to dry in the air.

Fixing.—My experience has been that the best results are to be obtained by placing such films of the sediment upon a heated copper plate and permitting them to remain at the proper temperature—point where the water boils when dropped upon the plate —for from one-half to one hour (see *Fixing of Blood*, page 74). Fairly good results are to be obtained, however, by placing the slide in a slide-forceps and passing it directly through the flame several times. It is necessary to keep the slide as hot as it can be borne to touch the palmar surface of the hand for at least three minutes. Whenever feasible, the former method is to be employed.

Staining.—At least one specimen film from the sediment of every fluid should be stained for the tubercle bacillus (see *Sputum*, page 438). Stain a second with anilin-gentiān-violet and continue with Gram's method (see *Gonococcus*, page 494). It is my custom to stain a third slide with an alcoholic solution of eosin for one-half minute and Delafield's hematoxylin for three minutes; and one slide with a saturated alcoholic solution of sudan III for from one to three minutes for the detection of fats which are stained a pink with this reagent; a knowledge of the presence of fats is also gained by staining with a solution of osmic acid, which stains such substances black. The eosin is employed for the detection of cells showing eosinophilic granules, such leukocytes being rather common in pleural and peritoneal fluids.

Cytodiagnosis consists in a differential study of the cellular elements found in such fluids. An excess of lymphocytes is probably suggestive of tuberculosis; while a preponderance of polynuclear cells points toward suppurative processes. In transudates, endothelial cells are found in sheets and clusters. In cancerous involvement of the serous surfaces one would expect to find many endothelial cells in the effusion from involved sacs.

Transuded fluid will be found to contain a few leukocytes and endothelial cells, many of which show evidence of fatty degeneration. In ascitic fluid during the course of myelogenous leukemia many eosinophils and mast-cells have been noted. In cases in which the fluid is concealed for a long time crystals of cholesterin and Charcot-Leyden crystals may be found, the former being usually found in hydrocele fluids.

Renal Cysts.—The fluid of hydronephrosis is readily distinguished from transudate fluid by both chemic and microscopic study. The specimen should be examined at once for urea, and if that substance is present in large quantity, an opinion as to its renal origin can be formed. While the presence of urea would serve to distinguish between the fluid from a renal cyst and ascites, it does not serve the same purpose in case the diagnosis lies between an ovarian cyst and a cyst of the kidney. Urea is at times present in the fluid of the former in fairly large amounts. Uric acid is present in the fluid of a kidney cyst, but it may too be found (traces) in the fluid from an ovarian cyst. The presence of epithelial cells from the uriniferous tubules is regarded by some authors as distinctive, but personally I should hesitate to make a diagnosis upon such a finding.

Ovarian Cyst.—The general appearance of fluid from ovarian cysts is very inconstant, but there is one feature which serves to distinguish it from the fluid of the majority of cysts—its high specific gravity. This is usually above 1.020, 1.023 to 1.025 being quite common, although I have seen such a fluid with a specific gravity of 1.010. The fluid from an ovarian cyst shows no tendency to coagulate and is of an alkaline reaction.

The large quantity of tissue débris present is rather characteristic of the fluid from ovarian cysts, although this finding is by no means reliable. If a hemorrhage has occurred within the cyst, the general findings are materially altered, and the fluid may be bloody or it may present a chocolate color. The cellular elements also may be distorted, and at times colloid-like bodies have been detected in the sediment. These colloid-like bodies are said to be found in connection with colloid cysts; but microscopic examination of the fluid is not of itself a reliable means of determining the variety of the cyst. The fluid from an ovarian cyst will be found to contain, in addition to the elements just named, hair, crystals of hematoidin, fat, and cholesterin. Chemically the fluid from ovarian cysts contains a variable amount of albumin and metalbumin.

Metalbumin Test.—Place 10 c.c. of cystic fluid in a test-tube and add 30 c.c. of alcohol; shake well and allow to stand for twenty-four hours; filter, squeeze out the precipitate, and suspend it in water; filter again. The second filtrate is opalescent, becomes turbid on boiling, but does not form a distinct precipitate. Acetic acid does not induce a precipitate, while acetic acid and potassium ferrocyanid produce a thick yellowish fluid. Millon's reagent gives a bluish-red color (see page 217). Concentrated sulphuric acid produces a violet color, as does glacial acetic acid. According to Huppert, when the fluid contains metalbumin, it yields a reducing substance when boiled with sulphuric acid. Metalbumin is also a constituent of other pathologic fluids.

Hydatid Cysts.—Fluid obtained by paracentesis from a hydatid cyst is, as a rule, clear and of an alkaline reaction, and has a specific gravity of 1.004 to 1.010. Such fluid often contains a small amount of grape-sugar and of albumin, and a larger amount of inorganic salts. Inosite and succinic acid are occasionally formed.

Hooklets.—The characteristic clinical features of the fluid from a hydatid cyst are the hooklets, the scolices, and the shreds of faintly yellow membrane (Figs. 122–125). The inner surface of this membrane is faintly granular and shows transverse striations. The individual scolex is best described by the illustrations, as are also the echinococcic hooklets. It is to be borne in mind that a positive diagnosis of hydatid disease is attained only through the detection of these elements.

Pancreatic Cysts.—The fluid from a pancreatic cyst is usually bloody. It varies in specific gravity from 1.010 to 1.030. Methemoglobin and crystals of cholesterin are often present. The chief proteid is serum-albumin. Pancreatic cyst-fluid contains a diastatic ferment, though this is not a feature characteristic of the pancreatic juice. The presence of the reactions for maltose in a fluid obtained by abdominal puncture is always strongly suggestive of cystic disease of the pancreas.

Fistulous Secretions.—The secretion from any abnormal channel varies with the viscus or the set of glands that the fistula drains. In analyzing such secretions it is necessary to examine them for the various body-fluids.

EXUDATES.

An exudate may be serous, seropurulent, chylous, purulent, hemorrhagic, or putrid.

SEROUS EXUDATES.

Exudates composed for the most part of serum are clear, pale, or of a light straw color, and display a specific gravity not greater than 1.008. See *Specific Gravity of Transudates*, p. 488. When serous exudates are permitted to stand, they develop a whitish color and more or less fibrinous coagula.

Microscopically such exudates may contain few red corpuscles, polynuclear leukocytes, and when derived from the serous sacs, endothelial cells showing areas of fatty degeneration are to be found.

Generally speaking, exudates display a higher specific gravity than the transudates, and when there has been added to a serous exudate a variable amount of fibrinous material, the exudate is referred to as serofibrinous in character.

Inoscopy.—This method for the study of inflammatory exudates recovered from the serous sacs has recently been described by A. Jousset, and consists in the digestion of the fibrinous coagula of such fluids with the following fluid:

Pepsin	2 gm.
Glycerin	10 c.c.
Hydrochloric acid	10 c.c.
Sodium fluorid	3 gm.
Distilled water	1000 c.c.

The digested coagulum is now sedimented, and such sediment stained and examined for tubercle bacilli. Jousset used the sediment for the inoculation of animals. It is claimed for inoscopy that the exudate thus treated is more likely to excite tuberculosis when injected into animals than is the fresh sediment from such exudates.

SEROPURULENT EXUDATES.

The term seropurulent is employed to designate an exudate in which there has been the admixture of a moderate number of leukocytes and pus-cells.

CHYLOUS EXUDATES.

Exudates of chylous and chyloid fluids are to be found in the serous sacs, most frequently in the peritoneal cavity, and less commonly in the pleural cavities; they have been found in the pericardium. Chylous exudates, like other exudates, will be found to contain sugar, and, as a rule, more than a mere trace is to be detected.

Characteristics.—These exudates are of a milky appearance. When they are chyloid, they bear a close resemblance to pus. The decided turbidity of these fluids is due to the presence of fat-globules, as is shown by microscopic examination. I have studied two cases of chylous fluid obtained from the peritoneum in which no chyle was present.* It has been shown by various observers (Lion) that in chyloid fluids the turbidity depends upon the presence of albuminous substances (nucleo-albumins), and degenerated epithelial cells may account in part for the turbidity of chylous exudates.

For distinctive features and *Clinical Significance of Chylous Exudates*, see p. 537.

HEMORRHAGIC EXUDATES.

A hemorrhagic exudate is essentially serofibrinous; it differs from that commonly known as serous exudate only by the admixture of red blood-cells. Microscopically, such an exudate will be found to contain a profusion of red blood-cells, polymorphonuclear leukocytes, a few endothelial cells, and cholesterin crystals. Hemorrhagic exudates collect in the pleuræ and in the peritoneal cavity as a result of tuberculosis, carcinoma, sarcoma, and cirrhosis of the liver. (See *Cytodiagnosis*, page 534.)

PURULENT EXUDATIONS.

Pus is a turbid fluid of a grayish-white or greenish-yellow color and of an alkaline reaction. It varies greatly in its consistence and specific gravity. When spoken of as an exudate, we mean that pus has accumulated in one of the natural cavities of the body. Occasionally we refer to an effusion of pus in the tissues (phlegmon); and at times to pus secreted from the free surface of a wound as an exudate.

When collected in a conic glass and allowed to stand in a cool place, pus separates into two layers—the upper layer is of a light-yellow color and of a watery consistence, while the lower layer is creamy, opaque, and contains the pus-cells. The admixture of blood or its pigment may give a brownish or brownish-red color to the latter layer. Putrid pus is, as a rule, liquid, of a greenish or brownish-red color, and emits a decided odor of indol and skatol.

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* "Journ. Amer. Med. Ass.," Feb. 18, 1905.

Microscopic Study.—When examined under the microscope, pus may be found to contain crystals of cholesterin, hematoidin, crystals of fatty acids, degenerated leukocytes, giant pus-corpuscles, pus-cells showing deposits of fat, red blood-cells, and débris. Pus thinly smeared upon a slide, fixed by heat, and stained with the ordinary anilin dyes will be found to contain bacteria in the majority of instances, but occasionally bacteria-free pus is to be encountered.

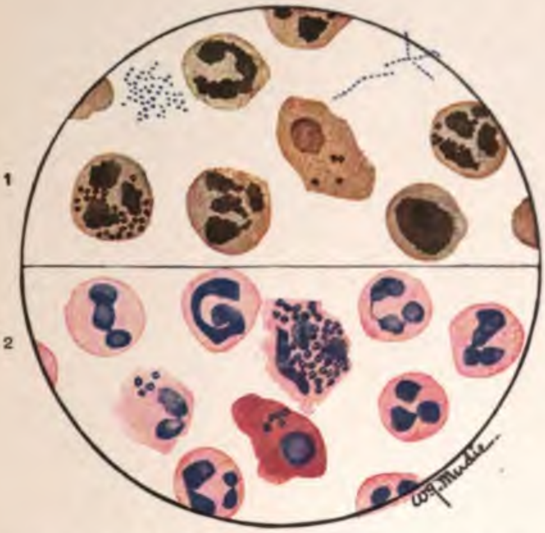
Staining.—At least one slide from all specimens of pus should be stained for the tubercle bacillus. Stain for twenty-four hours with a weak solution of carbolfuchsin (see *Sputum*, page 438), since this organism commonly figures in the production of suppurative maladies. Although it is questionable whether or not the tubercle bacillus of itself is capable of inducing suppuration, experience shows that it is often associated with the pus-producing organisms. Bacteria-free pus, when obtained from a serous cavity, should be diluted, say, ten to twenty times, with sterile water, and a small quantity of the mixture injected into the peritoneum of either a rabbit or a guinea-pig. In this way it is at times possible to establish a positive diagnosis when the microscope has failed to detect the tubercle bacillus.

Gonorrhea.—Inflammatory processes of the male urethra are, as a rule, of bacterial origin. The gonococcus is usually the pioneer infecting organism, which by its development reduces the resisting power normally present in the urethral mucosa, thus rendering this surface more susceptible to the development of other pathogenic bacteria.

A few drops of a serous exudate may escape from the meatus before the characteristic creamy discharge of gonorrhea is observed. It is possible to make a diagnosis of gonorrhea in its earliest manifestation by examining cover-glass preparations of this initial serous discharge.

Microscopic Study.—Direct the patient not to void urine for one or more hours before he presents himself for examination. When he is seen, have him pull back his prepuce; place the finger between his testicles, and then gradually draw it forward. By this means pus will escape from the meatus. Touch this drop

PLATE 34.



1. Urethral pus stained by Gram's method; note gonococci stained brown and situated in pus-cells and epithelial cells; staphylococci and streptococci stain violet (obj. B. and L. one-twelfth oil-immersion).

2. Urethral pus containing gonococci and stained by Small's method (obj. B. and L. one-twelfth oil-immersion).

of pus with a tooth-pick or with the edge of a slide (see *Method of Making Smears*, page 35), and smear it thinly over nearly the entire length of a microscope slide. Fix by passing it through the flame, care being taken lest too great a heat be applied.

The specimen is now ready for staining the gonococci, all of which stain readily by any of the anilin dyes. Eosinophilic leukocytes are often present in gonorrheal pus, but they bear no fixed proportion to the number of such cells found in the blood. The percentage of eosinophilic cells in the pus, however, is not in excess of the percentage found in the blood (see *Cytodiagnosis*, page 490). Gonococci are seldom found within the eosinophilic cells. Mast-cells are unusual.

For practical laboratory work I have adopted the method devised by my assistant, Dr. J. Hamilton Small, and I have found that this plan of staining gives as satisfactory results as the other more complicated methods.

Small's Method.—After the pus has been smeared and fixed in the usual manner, proceed as follows:

1. Place in a slide-forceps and stain for thirty seconds with a 2 per cent. aqueous solution of methylene-blue.
2. Wash in a 10 per cent. solution of sodium hydroxid.
3. Pour off the sodium-hydroxid solution and immediately follow with carbolfuchsin (dilution 1–10), and allow to stain for fifteen seconds.
4. Wash in water, dry, and mount.

By this method the general field will be stained pink; the nuclei of the leukocytes, pus-cells, and epithelial cells are stained a dark blue. The gonococcus stains a brilliant blue (Plate 34), while other pus-producing bacteria stain a less intense blue.

Methylene-blue.—Add to the specimen a few drops of an aqueous solution of methylene-blue (2 per cent.), and allow to stain for from two to three minutes without the addition of heat. By this method the gonococcus will be found to stain a deep blue, while the pus-cells and the epithelial cells are less intensely stained. Other bacteria, such as the staphylococcus and the streptococcus, when present, are also stained, but the staphylococcus is seldom arranged within the bodies of the pus-cells and is, therefore, readily differentiated from the gonococcus. By this stain it is not possible to distinguish the gonococcus from other cocci by its peculiar diplococcus (biscuit) formation.

Gram's Method.—1. To a fixed specimen of urethral pus add a few drops of anilin-gentian-violet; heat to steaming, and allow to stain for fifteen seconds.

2. Wash in water, and without drying—

3. Add a few drops of Gram's iodin solution and permit to remain for from one to two minutes.

4. Wash the specimen in 70 per cent. alcohol until but a faint violet color remains.

5. Stain for two minutes with a saturated alcoholic solution of Bismarck-brown. Wash in water, dry in the air or by heating gently, mount in Canada balsam, and examine under an oil-immersion objective.

By Gram's method the gentian-violet stains all bacteria present, but after treatment with Gram's solution of iodin and alcohol the gonococcus is decolorized, while streptococci, staphylococci, and other diplococci remain violet. The addition of a second stain—Bismarck-brown—stains the previously decolorized gonococcus. Thus, by this method, the gonococcus is stained a light brown, while other pathogenic bacteria are stained violet. The nuclei of the pus-cells and of the epithelial cells stain a mahogany color, while the bodies of these cells stain a less intense brown.

Streptococci.—In a few instances I have been able to study urethral pus in which the general symptoms resembled those of gonorrhea, except that the urethritis subsided in a shorter time, and in which streptococci only were present. In a case studied at the Medico-Chirurgical Clinic, through the courtesy of Dr. Leon Gans, I was able to obtain streptococci only from both scrapings and cultures made from a small cervical ulcer from the woman with whom the patient had been cohabiting. Careful cultural studies of the streptococcus obtained from the cervix and that obtained from the urethral pus of the man showed these organisms to be practically identical. Furthermore, inoculations showed them to be highly pathogenic for animals. This patient finally developed orchitis involving both testicles.

Streptococci and staphylococci appear in the urethral discharge during acute gonorrhea coincidentally with improvement of the symptoms. In fact, I have grown to regard the appearance of the streptococcus as a precursor of the disappearance of the gonococcus from the urethral pus. Indeed, it is rare to find gonococci when the pus contains many other bacteria. One often sees specimens of urethral pus from cases of chronic urethritis in which streptococci, staphylococci, extracellular diplococci, bacilli, and diplobacilli are to be found in great numbers. Occasionally, in such specimens, extracellular diplococci may be found, the staining reaction of which, by Gram's method, is characteristic of the gonococcus. In my experience these organisms have not proved to be gonococci, and I do not recall ever having found gonococci when the above-named organisms were numerous.

Urine.—It is impracticable to search for the gonococcus in urinary sediments. My experience differs widely from that of many writers, since I have been unable to recover the gonococcus from this source, even though the urinary sediments contained considerable urethral pus, and although the pus collected from the urethra showed many gonococci.

Glanders.—In the cutaneous form of glanders pus collected from the abscesses will be found to contain bacilli known to this disease. The Bacillus mallei may also be recovered from the nasal secretions and from the blood.

Anthrax.—Pus collected from the abscesses and serum from the vesicles of anthrax commonly contain many large segmented bacilli (Fig. 207). These bacilli are readily stained by the anilin dyes, so that their detection is most simple.

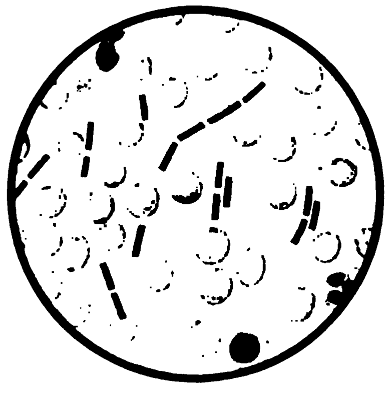


Fig. 207.—Bacillus of anthrax in blood of subject who died of the disease (Jakob).

Tetanus. — The exudate from the initial wound of tetanus is often found to contain many bacilli clubbed at one extremity.

Detection.—Secure scrapings from the wound or from the tissues immediately surrounding the site of the initial puncture, smear these scrapings thinly upon a cover-glass, fix by heat, and stain for from one to two minutes with a solution of carbolfuchsin, or with a 2 per cent. aqueous solution of methylene-blue.

Leprosy.—Lepra bacilli are often to be found in the exudates from suppurative surfaces. They are also to be detected by incising a small lepra tubercle, compressing it firmly, and smearing the exudate upon a slide (see *Staining of Tubercle Bacillus*, page 438).

Animal Parasites.—The Amœba coli (page 388), Balantidium coli (page 395), and certain protozoa may be encountered in purulent exudates.

Filaria.—The Filaria sanguinis hominis may collect in the tissues and produce a large chylous exudate which in time makes its way to the surface and appears not unlike an abscess (see *Guinea-worm*, page 149). Living filaria are occasionally found in this exudate. The echinococcus (page 404) and Schistosoma hæmatobium (page

295) are occasionally seen in abscesses of the liver, lungs, etc., and have already been discussed at length. The trypanosoma may also be found in the exudate recovered from ulcerating surfaces (see *Trypanosomiasis*, page 151).

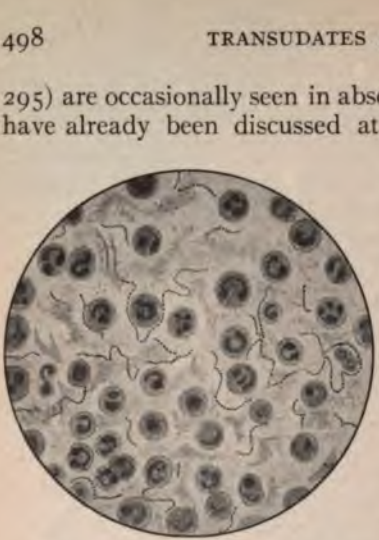


Fig. 208.—Streptococcus pyogenes from a case of empyema secondary to pneumonia (Jakob).

Micrococci.—Pus from whatever source is likely to contain cocci, among which the Streptococcus pyogenes (Fig. 208), the Staphylococcus pyogenes (Fig. 209), the pneumococcus, the Diplococcus intracellularis (meningococcus) (Fig. 213), the gonococcus, and the Micrococcus tetragenus are the most common. The Bacillus pyocyaneus, the Bacillus coli communis, the bacillus of Friedländer, are also frequently found in pus, and it is the rule to find many other bacteria in purulent exudates, the characteristics of which it is not necessary to discuss at length. (For the methods for the determination of questionable organisms recovered from pus the reader is referred to special works on the subject of bacteriology.)

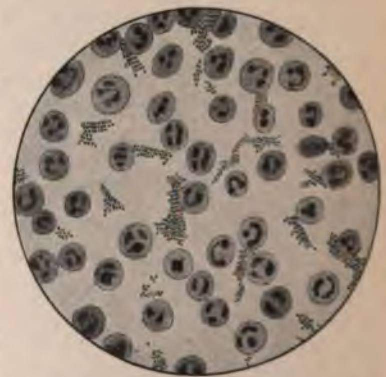


Fig. 209.—Staphylococcus pyogenes albus from an abscess of the parotid gland (Jakob).

Fungi.—Pus resulting from the development of the actinomyces (Fig. 183) is occasionally to be recovered from abscesses in various portions of the body. These abscesses develop most often in the structures adjacent to the buccal cavity, and have also been described in relation with the genito-urinary tract and the serous surfaces. Van der Veer regards thoracic, pulmonary, and abdominal actinomycosis as being a common occurrence, and pus from the thoracic and abdominal regions should always be examined for the actinomyces. Cutaneous lesions have occasionally been observed. A. Poncet has reported in detail 146 cases of actinomycosis hominis collected from the various portions of France. Erving* reports six cases, and in addition reviews the literature on American actinomycosis.

Detection.—Pus from the lesions of actinomycosis usually contains small kernels or nodules. Place one of these nodules upon a slide and crush it by pressure applied to a heavy cover-glass or a second slide; then examine the specimen (unstained) under a one-fourth or a one-sixth objective for the presence of the ray-like formation of the fungus (Fig. 183). Actinomycotic pus may be free from bacteria, although the finding of cocci and bacilli in such pus should not discourage a careful search for the actinomyces.

This fungus may be cultivated upon the ordinary culture-media at incubating temperature, yet in the majority of instances cultural studies are not required. During my service at the Philadelphia Hospital (1904) I studied a case in whose sputum the actinomyces was first found by my colleague, Dr. Rosenberger.

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* "Johns Hopkins Hosp. Bul.," Nov., 1902, p. 261.

CHAPTER XI.

CEREBROSPINAL FLUID AND SYNOVIAL FLUID.

CEREBROSPINAL FLUID.

THE study of the cerebrospinal fluid often furnishes valuable information in connection with both cerebrospinal affections and in differentiating meningitis from other conditions closely resembling it. This fluid may contain animal parasites (see *Trypanosomiasis*, page 151).

Collection of the Fluid.—After the age at which the cranial bones have united lumbar puncture becomes necessary in order to withdraw the cerebrospinal fluid; but early in life a needle may be thrust through the anterior fontanel and fluid withdrawn from the lateral ventricles.

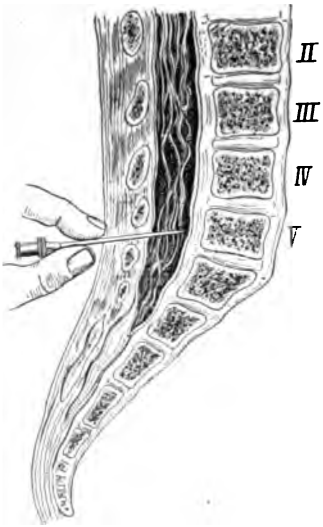

Fig. 210.—Anatomic preparation from a child twenty-one months old, showing location for lumbar puncture between third and fourth spinous processes (Fruhwald).

Lumbar Puncture.—Place the patient upon his side, flex the superior thigh upon the abdomen, and then cleanse and render the skin of the lower thoracic and lumbar regions aseptic. The site of puncture should be on a level with the junction of the third and fourth lumbar vertebræ at a point one-half inch to the right or left, *above the median line*, depending upon which side the patient is lying (Figs. 210, 211, 212). Anesthetize the skin by spraying with ether. Select a small needle, from four to six inches in length, with which to make the puncture, since it is often more than three inches from the surface of the skin to the spinal canal. Place the index-finger firmly upon the needle, as a guide to the depth of the puncture, and thrust it obliquely through the tissues at such an angle that the point of the needle will pass between the laminæ of the vertebræ. It is not necessary to attach a syringe to the needle,

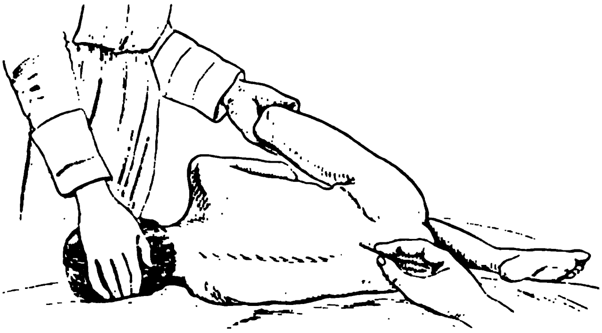

Fig. 211.—Method of inserting needle in lumbar puncture—child in lying posture.

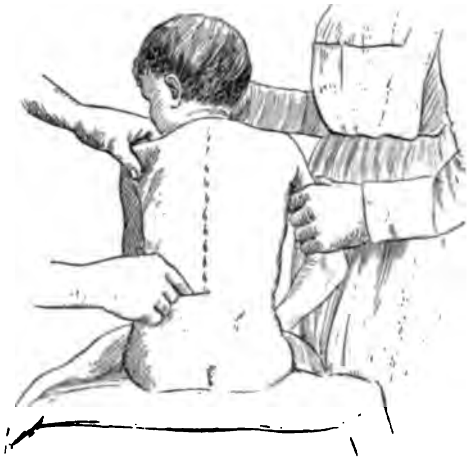

Fig. 212—Method of introducing needle in lumbar puncture—child in sitting posture.

because the spinal fluid will escape as soon as the needle enters the cerebrosubarachnoid space.

Allow a few drops of the fluid to flow directly into a sterile test-tube. This tube is then corked and placed in an incubator at a temperature of 37° C. (98.6° F.). It is well to make cultures from this fluid upon Löffler's blood-serum, bouillon, agar-agar, and glycerin-agar. The tubercle bacillus when present will develop upon the glycerin-agar within from ten days to two weeks. The Diplococcus intracellularis, the pneumococcus, and the other pathogenic organisms will be found to develop appreciable growths in from twenty-four to forty-eight hours when kept at 37° C. (98.6° F.).

As the fluid continues to flow drop by drop it should be collected upon several clean slides, dried in the air, fixed by heat, and stained. (See *Cytologic Study*, p. 538.)

Staining.—Cerebrospinal fluid should always be stained for the tubercle bacillus (see page 438) by Gram's method for the detection of the Diplococcus intracellularis, and also to determine whether or not certain bacilli present are decolorized through this method of staining (the bacillus of Friedländer and the colon bacillus decolorize). Stain a third slide with a 2 per cent. aqueous solution of methylene-blue. The same routine should be followed in the staining of bacteria cultivated from the cerebrospinal fluid. It is not within the scope of this volume to deal with the cultural characteristics of the different bacteria known to invade the meninges, and for such knowledge the reader is referred to special works on bacteriology. Microscopically the normal cerebrospinal fluid will be seen to contain a few leukocytes and a small amount of granular matter; but when the fluid is purulent, it differs in no way from the pus found in any other of the serous sacs.

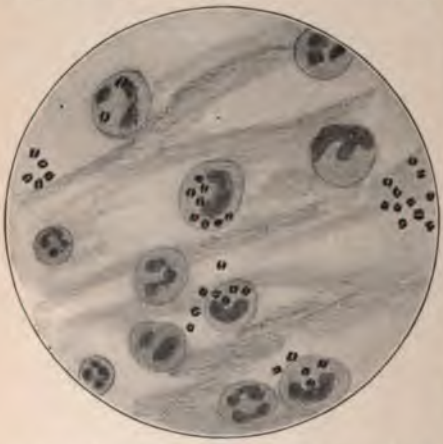

Fig. 213.—Diplococcus intracellularis. Meningeal pus obtained by lumbar puncture from case of epidemic cerebrospinal fever observed at Philadelphia Hospital (obj. Spencer one-twelfth oil-immersion).

Diplococcus Intracellularis.—This organism was first described by Weichselbaum, and later studied at length by Councilman and Mallory and Wright of this country. It is generally conceded to be the exciting cause of epidemic meningitis. The characteristic situation of this diplococcus is within the bodies of the pus-cells and the leukocytes, but many extracellular diplococci are always present (Fig. 213). The situation of the cocci within the pus-cells and the fact that they are not stained by Gram's method are features displayed by the Diplococcus intracellularis and the gonococcus. The diplococcus of meningitis, however, grows readily upon the ordinary culture-media, and this serves to distinguish it from the gonococcus, which is grown upon a special medium only.

Personally I have recovered from the meningeal exudate the Bacillus tuberculosis, the colon bacillus, the Streptococcus pyogenes, the Staphylococcus pyogenes albus, the Staphylococcus pyogenes aureus, the pneumococcus, and the Diplococcus intracellularis meningitidis. Other observers have also found the Bacillus meningitidis purulenta and the typhoid bacillus.[*] In one case studied at the Philadelphia Hospital in which meningitis followed puerperal sepsis I recovered a capsulated bacillus from both the meninges and the uterine cavity at postmortem. The bacilli from both sources were identical in every respect and were highly pathogenic for guinea-pigs. In many respects this organism resembled the bacillus of Friedländer, but I hesitate to regard it as that organism, since in certain cultural studies it differed widely from the organism described by Friedländer. In a second case I recovered the Diplococcus intracellularis from the meninges antemortem and from the endocardium, the pericardium, and a myocardial abscess at postmortem. Through the courtesy of Dr. J. H. Lloyd a case was studied in which the meningeal pus contained only colon bacilli.

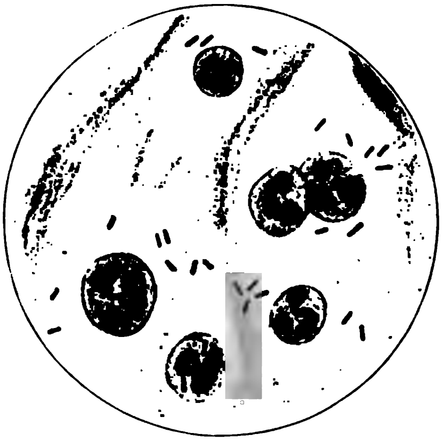

Fig. 214.—Meningeal pus containing colon bacilli. Observed at Philadelphia Hospital. Pus recovered at postmortem (obj. B. and L. one-twelfth oil-immersion).

In my experience it has been rather common to find the diplococcus of pneumonia in association with the Diplococcus intracellularis; and when this condition exists, certain of the diplococci are not decolorized by Gram's method.

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[*] C. Straubli, "Deut Archiv. f klin Med.," Bd. lxxxii, p. 90.

Galdi and Appani* have found a large amount of uric acid in purulent cerebrospinal fluids.

For cytologic study of spinal fluid see p. 538.

SYNOVIAL FLUID.

The study of the fluid from the serous sacs of the various articulations may provide valuable clinical knowledge in many instances, as is illustrated by the fact that when an arthritis is found to complicate either lobar pneumonia or gonorrhea, the specific diplococcus of these diseases may be recovered from the synovial fluid of the infected joints. The Diplococcus intracellularis is sometimes recovered from the synovial fluid, and the tubercle bacillus, which is known to invade these structures, may be found. Following traumatism and following lacerated or punctured wounds of the joint regions some one or more of the pus-producing organisms may be recovered from the synovial fluid and other bacteria may also be found. During the course of scarlet fever and of rheumatism bacteria may be recovered from the joint fluids of inflamed joints. Throughout England and America the belief is quite strong that acute articular rheumatism is probably excited by a coccus—"micrococcus rheumaticus." Beattie,† after a series of experiments, concludes that the "micrococcus rheumaticus" is a distinct microbe, and an etiologic factor in acute articular rheumatism. The researches of Cole, Jachmann, and of the author fail to confirm the findings of Beattie, since micrococci were not found in the fluid from infected joints or in the blood of persons suffering from acute articular rheumatism.

Collection.—The skin overlying the joint should be scrubbed well with water and green soap, and a wet mercury-bichlorid dressing should be applied for a few hours. It is my custom to wash the skin surface over the joint with ether just before the puncture is made. Then determine that there is a distinct fluctuation over the joint, and, placing the finger about one-half inch from the point of a previously sterilized needle, plunge it quickly into the sac. The point of puncture should not be on a level with the articular surfaces of the bones, but at least one-half inch from the joint cavity, so as to avoid all possibility of wounding the articular cartilages.

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* "Brit. Med. Journ.," Dec. 3, 1904 † "Riforma Med.," Dec. 14, 1904.

The technic for the making of cultures and cover-glass preparations differs in no way from that described in the chapter devoted to the study of the cerebrospinal fluid (page 500).

Cultures.—In certain chronic conditions in which there is an accumulation of fluid within the synovial sacs this fluid, when smeared thinly upon a slide and stained, will be found free from bacteria. When, however, from ten to thirty drops of such fluid are added to from 150 to 500 c.c. of bouillon and placed at an incubating temperature for forty-eight hours, a certain clouding of the bouillon is produced. Bouchard has described extremely minute organisms which develop from the fluid obtained from chronic rheumatic conditions. Through the courtesy of the late Dr. Frederic A. Packard I was privileged to repeat these investigations during my term of service at the Pennsylvania Hospital. The clouding of the bouillon described by Bouchard was observed in every instance, but I was unable to detect, with any degree of satisfaction, the miniature cocci to which this author has referred. I am, therefore, inclined to regard the change occurring in the bouillon as due to chemic changes rather than to bacterial development.

CHAPTER XII.

DISEASES OF THE SKIN.

Favus.—Honey-combed ring-worm, or Tinea favosa, is occasionally encountered in this country and is known to be common throughout Europe. It usually affects the scalp and the eyebrows; but it may involve the face and other portions of the body. In one instance, studied at the Philadelphia Hospital, a case in which the patient's entire scalp and genitalia had been involved, I recovered the fungus from a mycotic patch on the tonsil.

The disease is due to the infiltration of the epidermis and hair-follicles with the mycelium and spores of a fungus which is usually termed Achorion Schönleinii. This name, according to many observers, includes several species of the fungus. During the past three years five cases of favus have been treated at the dermatologic clinic of the Medico-Chirurgical Hospital. In all these cases the patients, who were of foreign birth, had probably contracted the disease before emigrating to this country.

Collection.—Remove one of the crust-like masses, cut it in half, and from near its center chip out a small portion. Place this portion of the mass on the center of a slide, add a few drops of water, and crush the specimen completely. Now spread the fragments in a thin layer on the slide, fix by heat, and stain by heating for from one to two minutes with carbolfuchsin; wash in water, dry, mount, and study under a one-sixth objective. The specimen will be found to consist almost entirely of granular débris, but in the thin portions of the field a few imperfectly formed, oval or partially rounded bodies may be seen, as well as ribbon-like segmented chains of the fungus. These ribbon-like particles (mycelia) may be the only finding, and in certain cases they will be seen to divide dichotomously; they always show distinct segmentation, and are surrounded by a lighter area within which the fungus stains deeply. They are well seen under an oil immersion objective (Fig. 215). The fungus is stained less satisfactorily with methylene blue and the other anilin dyes.

A hair drawn from the infected area should be placed in a 10 per cent. solution of sodium hydroxid for from one-half to one hour, then placed on a slide, flooded with water, and covered with a cover-glass. By careful focus under a one-sixth objective it is at times possible to detect many threads and apparent spores of the fungus within the sheath of the hair-bulb. The infection of the hair is best demonstrated, however, by sectioning, and without such treatment I have found it difficult to demonstrate the presence of the fungus. Favus may, in rare instances, involve the stomach-walls, in which case this fungus may be found in the gastric secretion.

Tinea Tonsurans.—Tinea tonsurans is one of the most common of skin conditions in which clinical laboratory methods are of great service. The exciting cause is a small-spored fungus, Trichophyton microsporon or Microsporon Audouini.

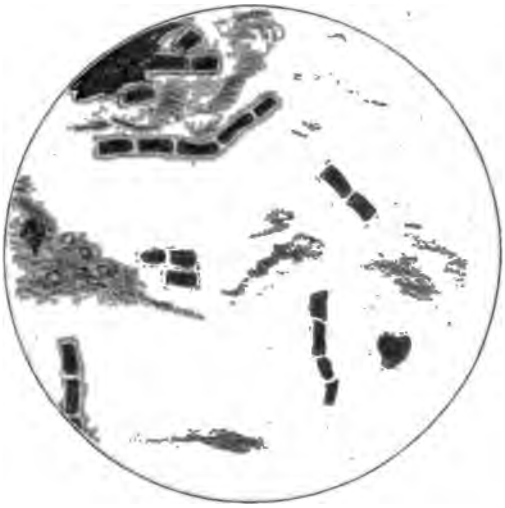

Fig. 215.—Tinea favosa, showing threads of the fungus; crusts from scalp of patient observed at Philadelphia Hospital (obj. B. and L. one-twelfth oil-immersion).

Detection.—Remove one of the diseased hairs, which will usually be found to vary from one-eighth to one-fourth of an inch in length, place the hair-stump and root in a solution of liquor potassæ, and allow to soak for one hour. Place the hair on a slide, flood it with water, and examine under a one-sixth or a one-eighth objective. The hair will be seen to be ensheathed by round bodies, which vary from 2 to 3 μ in diameter. These bodies are closely packed together, but by gently pressing a cover-glass on the hair they may be separated slightly; by rupturing the sheath, spores may be forced outside of the hair. Prolonged

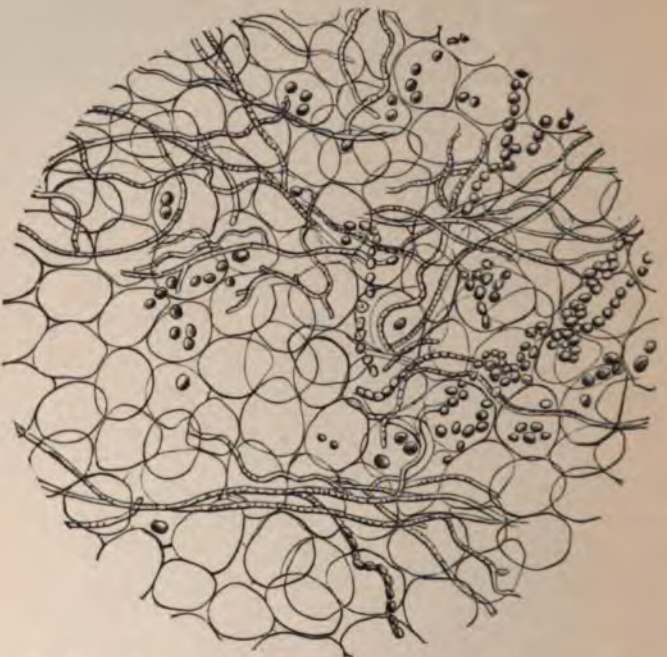

Fig. 216.—Epidermic scales from a case of squamous herpes tonsurans; potassium-hydroxid preparation; magnified 275 times (Eichhorst).

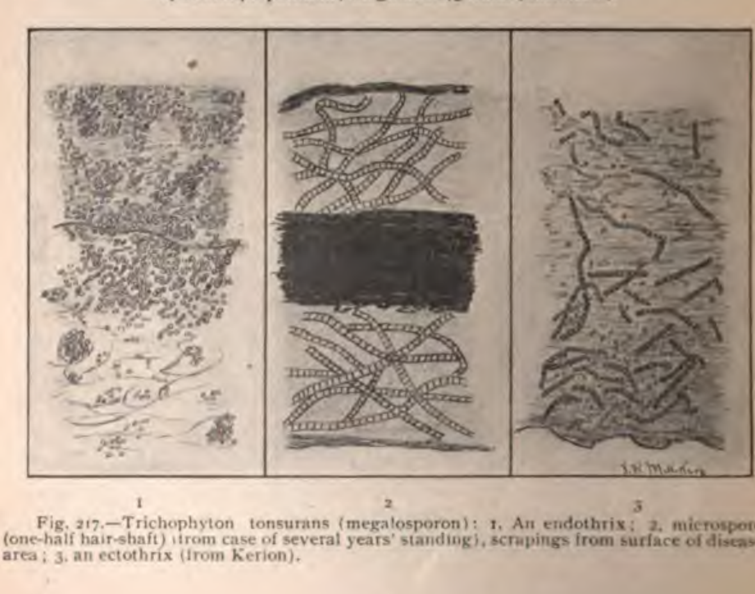

Fig. 217.—Trichophyton tonsurans (megalosporon): 1, An endothrix; 2, microsporon (one-half hair-shaft) (from case of several years' standing), scrapings from surface of diseased area; 3, an ectothrix (from Kerion).

soaking of the hair in liquor potassæ will enable one, by careful focusing, to detect mycelia surrounding the sheath (Fig. 217).

Tinea Trichophyton Endothrix.—The spores and mycelia of Tinea trichophyton endothrix (Fig. 217), as well as of Megalosporon endothrix and Megalosporon ectothrix (Fig. 217), may be detected in the manner described under *Tinea tonsurans.* A very small percentage of cases of ring-worm are caused by the latter of these parasites, and the fungus is commonly limited to the intrafollicular regions, though rarely mycelial threads extend above this portion.

It is at times difficult to differentiate these fungi, and personally I believe the diagnosis rests largely with the clinician. Cultural studies are recommended by some authors as giving a satisfactory method of distinguishing the different fungi known to play the part of the exciting cause of the ring-worm. It is not uncommon to find, in conjunction with ring-worm, small pustules, which are due to a secondary infection with pus-producing organisms.

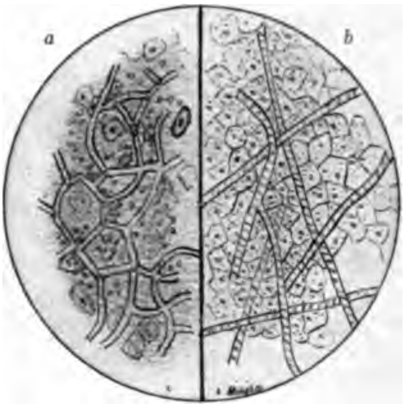

Fig. 218.—*a*, Tinea circinata (microsporon); *b*, trichophyton (megalosporon).

Tinea Circinata.—Patches of Tinea circinata occur over the body in more or less circular, solid, pale-red blotches covered with branny scales. Remove some of the scales and also extract a few hairs. Thinly smear the scales upon a slide, fix, stain, and examine for spores and mycelia (Fig. 218).

Tropic Tinea.—Tropic Tinea circinata may occur upon any portion of the body, and when studied microscopically, this disease will be found to be produced by a fungus, as indicated by the occurrence of many mycelia.

Tinea Barbæ.—Tinea sycosis (barber's itch) will be found, as a rule, to involve only the bearded portions of the body. It usually presents a folliculitis, with pustules which are distinguished from the coccogenic form of sycosis only by microscopic study. According to Saboraud's researches, suppurating Tinea sycosis is due to the Trichophyton megalosporon ectothrix (Fig. 219, *a*), which Crocker regards as an animal parasite. The parasite surrounds the hair, as do the parasites in the other forms of tinea (tonsurans). Remove the hair from the infected area, treat it for one hour with liquor potassæ, and examine it under a one-sixth or a one-eighth objective for segmented chains of the parasite.

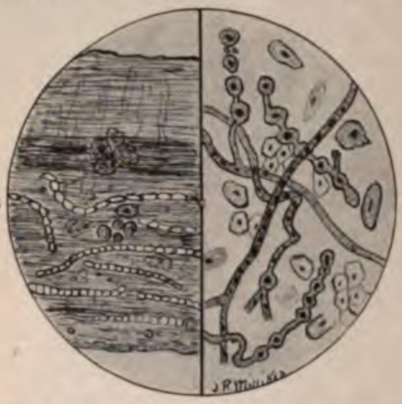

Fig. 219.—*a*, Tinea barbæ; *b*, Tinea cruris (after Crocker).

Mycotic Dermatitis (Dhobie Itch).—Under this name it is customary to include a number of varieties of erythema known to tropic districts. According to Mason, "dhobie itch" proper is induced by the Microsporon minutissimum; but recent observers have shown that many of the conditions regarded as dhobie itch result from other micro-organisms. P. Manson also believes that some forms of the disease are produced by the Microsporon furfur (Fig. 220, *a*) and by the trichophyton. Major Charles F. Mason* writes as follows: "There are at least three distinct forms of skin disease occurring in the regions indicated which are commonly known as dhobie itch: two of them are mycotic—those due to the Microsporon minutissimum and to the trichophyton, and one is bacterial—pemphigus contagiosus." These parasites are detected after the manner given for the Tinea sycosis.

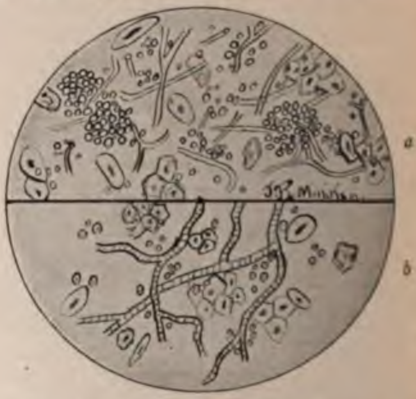

Fig. 220.—*a*, Tinea versicolor (Microsporon furfur); *b*, onychomycosis (Trichophyton endothrix of nails).

Both scrapings and cultures from the mycotic areas are liable to show bacteria, and the degree of irritation excited by their development is a moot question. In mycotic dhobie itch the scrapings from the festooned rings will be found to contain the trichophyton, "sometimes, however, when there is much inflammation, these elements cannot be found" (Mason).

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* "N. Y. Med. Jour.," Aug. 1, 1903, p. 221.

Onychomycosis.—Onychomycosis is a term applied to a condition in which either the Trichophyton endothrix or the Microsporon minutissimum is found to invade the nails (Fig. 220, *b*). The detection of the parasite in the nails is accomplished as already described.

"Gayle."—"Gayle" is a variety of puerperal sepsis which affects ewes during the lambing season, and which is known among sheep-breeders as a very fatal disease. Persons who skin animals dead of this disease are liable to infect their hands. The infection first produces a small pimple, which soon enlarges and fills with bloody serum. Klein[*] has obtained from these blebs and vesicles an organism which he found to be the exciting cause of the infection, to which he gave the name "Staphylococcus hæmorrhagicus." The organism produces hemorrhagic edema when cultures are injected into animals.

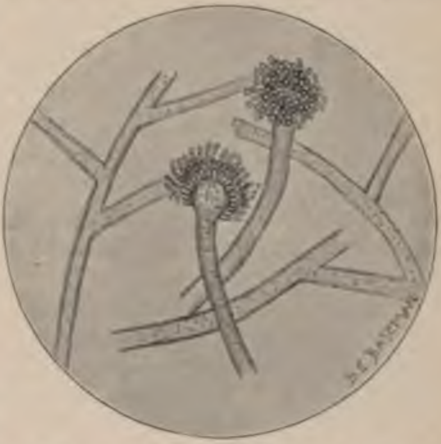

Fig. 221.—Mycelia and spores of fungus recovered from scrapings of the pigmented patches in pinta (after Montoya y Florez).

Tinea Versicolor.—Pityriasis versicolor is a parasitic disease which usually involves the skin of the trunk and chest, and is characterized by patches of a brown color. The detection of the Microsporon furfur is accomplished in the manner described under *Tinea tonsurans*. This parasite is one of the most characteristic fungi of the skin; its conidia are arranged in closely crowded, conic aggregations surrounded by the mycelia (Fig. 221). These conidia are larger, as a rule, than are those of the other forms of ring-worm. The mycelia are short and often unbranched; they may show segmentation and nuclei at regular intervals. Budding is not uncommon.

Detection.—A method suggested by Crocker is to place scrapings from the patch on a glass slide, wash with ether to remove the fat, and, when the mass is spread thinly, flood with liquor potassæ and examine under a cover-glass with a one-eighth or a one-twelfth objective.

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[*] "Brit. Med. Jour.," Aug. 4, 1897, p. 385.

Erythrasma.—Erythrasma is a vegetable parasite disease which produces brownish patches upon the skin.

Pinta.—Pinta is a tropic vegetable disease causing mycotic growths upon the skin. Place scrapings from one of the mycotic areas upon a slide, treat with liquor potassæ for one-half hour, and examine under a cover-glass with a rather high-power objective. Many dichotomous filaments, which are, as a rule, fine and cylindric, but at times granular and beaded, are seen. In places the mycelia form a dense net-work. This fungus is probably closely allied to the aspergillus.

Blastomycetic Dermatitis.—A number of cases of this disease have appeared in the United States, and it has also been observed in foreign countries.

Detection.—Remove a bit of a tissue from the advancing margin of the disease, and macerate for twenty-five minutes in a solution of sodium hydroxid (25 per cent.) in order to destroy the blood-cells and bacteria. Pus taken from the small abscesses should be treated in a like manner. A precaution which I should strongly advise is to treat both pus and tissue with ether in order to remove all fats, since to the inexperienced eye oil-globules resemble yeast-cells very closely. After treatment with sodium-hydroxid solution the yeast-cells are seen as highly refracting bodies, varying from 5 to 20 μ in diameter (Fig. 222). Cells in the stage of budding are commonly found. A one-sixth or a one-eighth objective will be found most serviceable in the study of this fungus.

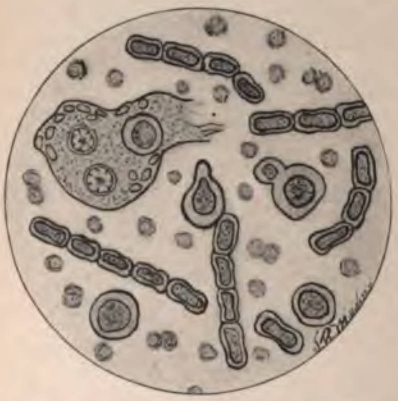

Fig. 222.—Blastomyces; different forms of the fungus; two budding cells; small bodies are leukocytes.

A closely allied fungous disease was first described by Wernicke in 1890, and has been studied by Gilchrist, of Baltimore. Ophüls and Moffitt, on the other hand, concluded that the disease is caused by a fungus which closely resembles that of blastomycosis. Five cases of generalized blastomycosis have been reported. The parasite has been found in pus and may appear in the sputum where the lungs are involved.

Mycetoma.—Mycetoma is a fungus growth of the foot known, especially in tropic districts, as "Madura foot." V. Carter detected a fungus in the black variety of this disease ("Chionyphe Carteri").

Detection.—Secure scrapings or a portion of the diseased tissue, macerate them in ether for twenty minutes, wash in sodium-hydroxid solution, and then smear upon a slide. Fix by heat, stain with one of the ordinary anilin dyes, wash in water, and examine under a one-sixth or one-eighth objective. The fungus appears not unlike that of actinomycosis, and displays many small round bodies. Mycelia which are sometimes clubbed may be found.

The mycetoma fungus grows extremely slowly in a hydrogen atmosphere, on a special medium composed of 100 parts of a 2 per cent. infusion of hay or potato; 9 parts of gelatin; 4 parts of glycerin; and 4 parts of grape-sugar. Both black and white fungi are at times recovered. An exhaustive description of this disease is to be found in Manson's "Tropical Diseases," third edition, page 680.

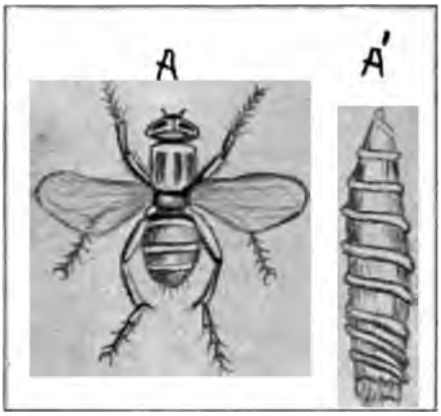

Fig. 223.—Screw-worm: A, Fly that deposits her ova on an open wound; A', larva American parasite (Blanchard).

Myiasis.—Myiasis is a disease produced by the screw-worm. The screw-worm (Fig. 223) is the larva of a dipterous insect, Chrysomyia mascellaria, common in certain portions of Africa and in North and South America. The adult female (Fig. 223) deposits her eggs on the surface of a wound, in the external auditory meatus, and in the nasal fossæ, from which ova the larvæ are developed. The larvæ are white, from one-half to three-quarters of an inch in length, and are formed of twelve segments. Each larva is circled with a spiral-like spine so arranged as to give the parasite a screw-like appearance (Fig. 223).

Clinical Location.—These larvæ are known to burrow through the tissues, destroying the mucous membrane, the muscles, and the cartilages. They may invade the serous sacs and the bones, producing extensive lesions. They have also been recovered from the interior of the eye and the conjunctivæ.

George Gray * has reported five instances in which the larvæ of the screw-worm were found in human beings. He further stated that the parasite is common in domestic animals and that it is widely distributed throughout America.

Vera Macaque.—Vera Macaque is the larva of an American insect (Dermatobia cyanicentris v. noxialis, Fig. 224). The larva shows many circular bands from which extend fine spines.

Impetigo Contagiosa.—During the past few years much discussion has arisen as to the etiology of impetigo contagiosa. On account of its prevalence among students, it has acquired the names of "foot-ball impetigo" and "scrum-pox." These small lesions, whether vesicular or pustular, are due to inoculation with pus cocci, which are introduced into the superficial layers of the skin.

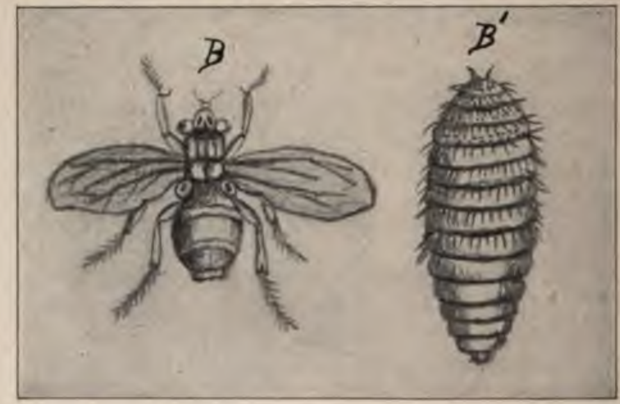

Fig. 224.—Dermatobia noxialis: an American fly. Ova are deposited into skin of animals and in man; B, Adult fly; B', larva (Blanchard).

Collection.—Collect either the pus or the serum directly from one of the lesions, smear it thinly upon a slide, fix by heat, and stain with carbolfuchsin, as for the tubercle bacillus, counterstaining with Gabbett's acid methylene-blue solution. The usual findings are streptococci, staphylococci, and diplococci. The streptococci are usually arranged in short chains of from four to six cocci each, although longer chains are at times observed. The clusters of staphylococci are almost equally common; but the diplococci are more rare. There seems to be a diversity of opinion as to the specific organism of this disease. It is stated that the impetigo contagiosa of Tilbury Fox is primarily vesicular and due to the streptococcus, while others consider it due to a specific micrococcus. The Staphylococcus pyogenes aureus and the

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* "Brit. Med. Jour.," Mar. 28, 1903.

Staphylococcus pyogenes albus are common findings, while the streptococcus of Fehleisen may occasionally be recovered from the vesicles. In the pustular variety and also in the vesicular form a mixed infection is not uncommon, and from my own observations both streptococci and staphylococci seemed to be capable of exciting the disease.

Erysipelas.—It is possible to recover bacteria from the serum contained in the blebs of erysipelas and also from the blood exuding through minute punctures made in the infected areas. The exudate, pus, and blood, when smeared on a slide, fixed, and stained will, in the majority of instances, contain streptococci; and Fehleisen has described a special streptococcus which he regards as the cause of this disease. I have isolated this streptococcus from a number of cases of erysipelas, and have succeeded in producing a similar condition in animals by inoculations. The Staphylococcus pyogenes albus, the Staphylococcus pyogenes aureus, and diplococci may also be found. Pfahler recovered only diplococci from a large number of cases of erysipelas which he studied at the Philadelphia Hospital. It was my privilege to examine many of the animals inoculated with this diplococcus, and the conditions excited by it were apparently identical with those caused by inoculation with the Streptococcus pyogenes and the Staphylococcus pyogenes. The above-mentioned bacteria may be cultivated from the skin overlying the erysipelatous areas. From a series of inoculations upon animals I am led to believe it possible for erysipelas to be excited by any one or several of the pus-producing organisms. The tissue changes excited by the diplococcus, the Streptococcus pyogenes, and the staphylococcus were apparently identical.

Eczema.—Probably one of the most important fields in which clinical laboratory studies have contributed to dermatologic diagnosis is the study of parasitic eczema. An admirable paper upon this subject appeared from the pen of Professor Jadassohn, of Bera.* Unna has long claimed that certain forms of eczema are caused by a micrococcus, and many investigators have found staphylococci in the lesions. This germ is not only associated with the impetiginous variety of eczema, but also with simple forms. By cultural studies the Staphylococcus pyogenes aureus, the Staphylococcus pyogenes albus, and, in some instances, the streptococcus may be detected.

While subject to numerous modifications, eczema appears like any ordinary wound infection; and since it is a disease of the derm, it is most likely to favor the development of the bacteria known to inhabit the skin. Unna conducted cultural studies of several cases, with the recovery of the following organisms: a staphylococcus which produced a white growth—the Staphylococcus pyogenes aureus; the Torula alba, and the Penicillium glaucum. The cultural features of these organisms have been described in detail.* In view of the fact that many varieties of bacteria must of necessity be obtained from the cutaneous surface, it is difficult to show conclusively that Unna's coccus of eczema is the exciting cause of the disease; and while it or some other specific organism may cause the initial changes, infection with other organisms must certainly follow before the disease has made much progress.

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* "Wien. med. Presse," Aug. 5, 1900.

Conditions of Bacterial Origin.—The Staphylococcus pyogenes aureus, albus, and citreus may be found either in pure culture or in conjunction with other bacteria in the following diseases, for which reason it has been deemed unwise to give the individual clinical appearances: Pemphigus contagiosa and pemphigus neonatorum; dermatitis papularis capillitii, and possibly other forms of pustular folliculitis; Quinquaud's folliculitis decalvans; impetigo of Bockhart, and other forms of impetigo sycosis (coccogenic and lupoid); boils, carbuncles, and abscesses; erysipelas, in which streptococci, staphylococci, diplococci, Pfahler's diplococcus, and the colon bacillus may exist; superficial whitlows; granuloma pyogenicum, and acne.

The Streptococcus pyogenes and the Streptococcus pyogenes of Fehleisen are found primarily in the following conditions: superficial whitlows, impetigo contagiosa, ecthyma, erysipelas, and erysipeloid processes. It is to be borne in mind that in any of these cutaneous lesions a mixed infection is common, and that bacilli are not infrequently detected in symbiosis with the pathogenic cocci. Furthermore, the lepra bacillus, the tubercle bacillus, and the bacteria of glanders, which have been considered elsewhere in this volume, may also be recovered from the individual lesions of these diseases.

Oriental Sore—J. H. Wright † has found that a peculiar parasite infests the tissues in tropical ulcers. There is yet some question as to the nature of these bodies. Christophers suggests the possibility of this being the Leishman-Donovan parasite (see page 525.)

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* "N. Y. Med. Jour.," Sept. 29, 1900, p. 547.

† "Jour. Med. Research," 1904, p. 472.

CHAPTER XIII.

MILK.

The mammary secretion may be obtained from the breasts of the human female during the period of gestation and of lactation. It usually makes its appearance in the mammary gland some time after the third month of pregnancy, after which it gradually increases in quantity, reaching its maximum about three days after labor.

COLOSTRUM.

Colostrum is a watery, slightly turbid fluid, which may be expressed from the breasts during the period of gestation, and, in rare instances, is to be obtained independently of gestation and even from the male breast. A variable quantity of this fluid is always secreted during the latter half of gestation and immediately after parturition, when it may be highly tenacious, resembling mucilage, and may vary in color from a pale-gray to an orange-yellow.

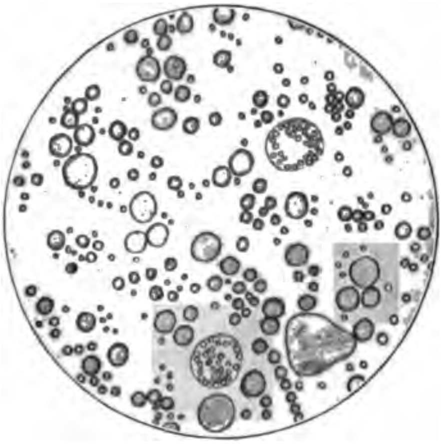

Fig. 225.—Colostrum from primipara. Fat-droplets, two colostrum corpuscles, and one epithelial cell (obj. Queen one-twelfth oil-immersion).

Distinctive Features.—The features that distinguish colostrum from milk are the color, density, tenacity, the high percentage of sugar and salts, and the property of the former of coagulating when heated to the boiling-point. According to Dunglison, the following chemic differences are known to exist between colostrum and normal milk:

	Colostrum.	Normal Milk.
Water	828	887.6
Fat	50	25.3
Casein	40	34.3
Milk-sugar	70	48.2
Ash	3	2.3

According to repeated analyses, casein does not appear in colostrum in appreciable amounts until after parturition.

Microscopy.—When examined microscopically, colostrum will be found to be composed of fat-droplets, leukocytes, epithelial cells, and the so-called colostrum corpuscles. The fat-droplets are the principal constituent and are usually quite small. The colostrum corpuscles are highly refractive, granular bodies, which vary greatly in size and are composed of many minute fatty granules (Fig. 225). Fixed specimens of colostrum when stained with sudan III or with osmic acid show the presence of fats.

HUMAN MILK.

The quantity of milk secreted in twenty-four hours varies between 500 and 1500 c.c. It is usually of a bluish color, alkaline reaction, and displays a specific gravity between 1.026 and 1.033 (the majority of specimens are near 1.030). Milk when subjected to a microscopic examination will be found to be a fairly homogeneous emulsion of fat, containing but few, if any, cellular elements. Dunglison gives Simon credit for the following table of comparison:*

	Human Milk.		Cow's Milk.		Goat.	Sheep.	Ass.	Mare.
	Variations.	Average.	Variations.	Average,				
Water	90.4 to 85.7	88.05	90.2 to 83.7	86.95	86.0	83.3	90.6	90.6
Casein and albumin	1.8 " 3.1	2.45	3.3 " 5.5	4.4	3.8	5.4	2.7	2.2
Fat (butter) . .	3.0 " 3.8	3.4	2.8 " 4.5	3.65	5.2	5.3	1.0	1.1
Lactose	4.5 " 7.0	5.75	3.0 " 5.5	4.25	4.3	5.2	5.3	5.8
Inorganic salts .	0.3 " 0.4	0.35	0.7 " 0.8	0.75	0.7	0.8	0.4	0.3

	Skimmed Milk.	Condensed Milk.	Butter.	Buttermilk.	Curd.	Whey.
Water	89.6	25.0	15.0	91.0	59.4	93.8
Casein and albumin .	4.2	14.0	2.2	3.7	27.7	0.8
Fat (butter)	1.0	10.0	82.0	0.8	66.4	0.3
Lactose	4.4	49.0	0.3	3.8	5.0	4.5
Inorganic salts . . .	0.8	2.0	0.5	0.7	1.5	0.6

Certain albumins, such as casein, lactoglobulin, and lactoalbumin, are found in milk. There are certain differences between the casein of human milk and the casein of cow's milk: first, it will be noticed that the casein coagula formed in human milk are smaller and less dense than are those seen to form in cow's milk. Human casein is precipitated by acids and salts, but it is less readily precipitated than is the casein from cow's milk. Again, human casein may not coagulate after the addition of a rennet ferment. Furthermore, human casein is precipitated by gastric juice, an excess of which causes dissolution. Hammarsten, in addition to the above points of difference, states that the two forms are not identical; but for practical clinical purposes their differences concern us but little.

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* Dunglison, twenty-third edition, p. 712.

Bacteria.—According to Welch, normal human milk is likely to become contaminated with the Staphylococcus epidermidis albus while passing from the acini of the gland through the lactiferous ducts and the nipple. It is to be borne in mind that this organism is constantly present in the skin, but that it is by no means the only bacterium common to cutaneous surfaces or known to invade the glandular portions of the skin; it appears, however, to be the only organism common to human milk. At times the Staphylococcus pyogenes aureus may be found in human milk.

Pathologic Milks.—Milk obtained from nursing women during the course of febrile attacks and during the existence of diseases associated with emaciation is likely to be deficient in fat and in lactose. During the course of jaundice biliary pigments of doubtful authenticity are said to have been recovered. In cases of malignant tumor of the mammary gland and after traumatism bloody milk has been observed. Microscopically, the milk obtained from a diseased breast is likely to show a large number of leukocytes, a finding most marked in connection with mammary abscess. A number of pathologic micro-organisms may invade the mammary secretion during disease—*e. g.*, during puerperal fever streptococci and staphylococci may occasionally be found. The pneumococcus has been recovered from the milk of nursing women suffering from lobar pneumonia, and in one instance, a case studied at the Philadelphia Hospital through the courtesy of Dr. George I. McKelway, I recovered a pure culture of the pneumococcus from the milk. In a second case studied the milk obtained from a woman suffering from typhoid fever gave a pure culture of a bacillus which agglutinated with the patient's blood (dilution 1–40); further tests for the Bacillus typhosus were not conducted. The milk of women suffering from typhoid fever will be found to agglutinate typhoid bacilli (see *Serum-diagnosis*, page 116).

Tubercle Bacilli.—Tubercle bacilli may appear in the milk of women suffering from tuberculosis. Roger and Garier* report the case of a young woman who was suffering from both pulmonary and laryngeal tuberculosis and who died seventeen days after delivery. The milk was found to contain tubercle bacilli, and the child which nourished upon the breast-milk for a few days eventually died of tuberculosis. Subcutaneous inoculation of a guinea-pig was also fatal, the animal developing tuberculosis which terminated fatally thirty-three days after inoculation.

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* "Sem. Méd.," Feb. 23, 1900, 20me Année, No. 9.

The milk of cows may be colored blue or green by the Bacillus pyocyaneus; and it appears reasonable that this organism or any other chromogenic bacterium when present in human milk would color that fluid.

It should always be borne in mind that the Micrococcus prodigiosus and the Bacillus prodigiosus produce a decided red growth, and that when the former of these organisms develops in milk, that fluid is liable to present a reddish color after it has been allowed to stand for a short time at room-temperature.

Detection of Bacteria.—Fill one tube of a centrifuge nearly to its top with the suspected milk, and fill the other tube with a dilution of one part of the suspected milk and three parts of sterile water; centrifugalize for five minutes; then pipet the sediment from each tube and place it upon separate slides, smear thinly, and fix by heat. Stain such smears with a 2 per cent. aqueous solution of methylene-blue and with carbolfuchsin and Gabbett's solution as directed for determining the presence of tubercle bacillus (see page 438). Both cocci and bacilli are usually found in this manner.

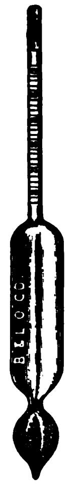

Fig. 226.—Quevenne's lactodensimeter.

SPECIFIC GRAVITY.

The specific gravity of milk is determined by the lactodensimeter of Quevenne. The instrument is standardized at a temperature of 60° F. (Fig. 226), so that a correction of the reading obtained at any higher temperature is necessary. The table accompanying each instrument should be consulted for this correction, and will indicate the true specific gravity when the temperature is between 46° and 75° F. This rather tedious process is readily avoided by heating the specimen of milk to a temperature of 60° F., when the Quevenne instrument will register accurately.

Author's Method.—The method I employ for this purpose is to place a thermometer in a glass jar containing warm water and to continue adding warm water to this jar until the temperature of the water reaches 60° F. The milk is then placed in a small glass cylinder (Fig. 227), which, with its contained milk, is stood in the warm water. The thermometer is then removed from the water, the mercury shaken down, and the temperature of the milk taken. When the milk reaches 60° F., the thermometer is removed and the lactodensimeter introduced. The specific gravity is read from the graduation on the instrument. This method has always appealed to me as most practical, and, since it necessitates but little extra work and no task of memory or reference to tables, I think it deserves recommendation to the profession. It is difficult to believe that the person designing the lactodensimeter had any other idea in mind, since the instrument was certainly designed for the purpose of simplifying laboratory methods.

BAUSCH & LOMB OPT. CO.

Fig. 227.—Plane cylindric glass.

ESTIMATION OF FAT.

The fat present in a given specimen of milk is most conveniently estimated by the lactoscope designed by Feser (Fig. 229). Fill the pipet with milk to the mark (M) and transfer this quantity of milk into the cylinder (C). Then attach a dropper to the tube at (P) and rinse the pipet thoroughly by forcing water through it, adding all the wash-water to the milk in the cylinder. The milk and wash-water are then shaken together, and more water is added slowly from a buret until the black lines on the glass plug (A) at the bottom of the cylinder become discernible. The cylinder is then held in a good light in order to view the graduated scales upon its right and left margins. The point on the right scale to which the mixture extends indicates the percentage amount of fat present; while the point on the left scale represents the amount of water in cubic centimeters that has been added.

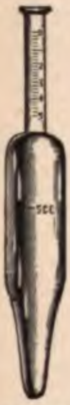

Fig. 228.—Milk tube. These tubes are used in place of the regular sedimentation tubes, and are graduated to give percentage of fats in milk according to the Leffman-Beam or Babcock method. Very useful for human as well as for cow's milk analysis.

All-important in the use of this instrument is that both the cylinder and the pipet be thoroughly cleansed after use.

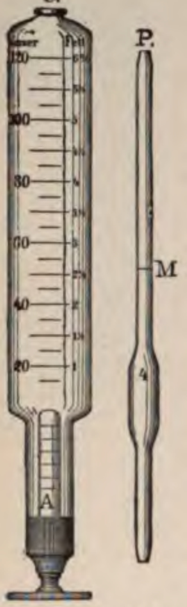

Fig. 229.—Feser's lactoscope.

ESTIMATION OF PROTEIDS.

Woodward's Method.—Two milk burets are necessary for this purpose. In each of these burets 5 c.c. of the milk to be analyzed are placed and the burets kept at a temperature of from 37° to 40° C. (98.6° to 104° F.) for from eighteen to twenty-four hours. At the end of this time the milk will be found to have separated into two layers: the superior layer is composed of a viscid, yellowish fat, while the inferior layer is composed of a more or less markedly opalescent fluid. A granular precipitate may also be seen at the bottom of the buret and clinging to the sides of the instrument. Now place both burets in a cool place and withdraw the milk-serum (inferior layer) from each buret into a separate tube graduated to 15 c.c., and add Esbach's reagent (picric acid, 10 grams; citric acid, 20 grams; water, 1000 c.c.) to the 15-c.c. mark. Stir the mixture in each tube thoroughly by means of a glass rod, and centrifugalize to a constant reading.

ADDENDA.

BLOOD.

TORKRITIZATION.

A clinical instrument introduced by H. E. Wetherill in 1894, and has been in use in my laboratory during the past few months. This unique and practical apparatus consists of a metal armature which is provided with two receptacles for the tubes. This armature is revolved upon a twisted cord (Fig. 230), and speed depends directly upon the length of the cord and the number of pulls per second. Wetherill claims that a speed of 25,000 revolutions a minute is readily obtained, and that the exact rate of speed is of little consequence since it is already too high to permit of error (see *Torfugation*, p. 528).

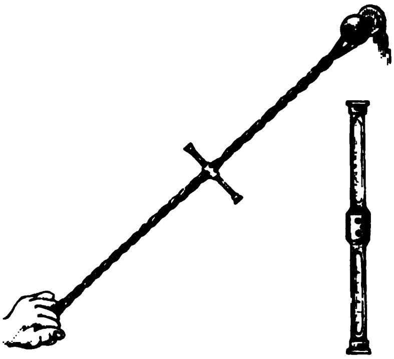

Fig. 230.—Wetherill's torkrit.

BIFF'S HEMOGELOMETER.

A test-tube, 25 centimeters long and 4 centimeters wide, is filled to one-half its depth with water. Provide this tube with a cork stopper containing two openings. Through one opening a thermometer passes and through the other a glass rod, at one extremity of which is a platinum wire coiled so as to form five loops, three millimeters in diameter, the one above the other.

Heat the water to 68° to 70° Fahr. Fill each loop by touching it to the summit of a drop of blood, and in from three to five minutes later place the glass rod into the cork and submerge the lower drop of blood, when, should it be diffused into the water, continue to immerse the successive loops at intervals until one of them is not diffused. Note the coagulation time. The time required for normal blood is from seven to ten minutes.

WETHERILL'S ANTE-MORTEM BLOOD COLORS.

This color-scale is a most ingenious one, and is doubtless an accurate test for the percentage of oxyhemoglobin (Fig. 231). It approximates more closely the color of the blood than any other printed scale I have been privileged to examine. The technic for its employment is given in each booklet.

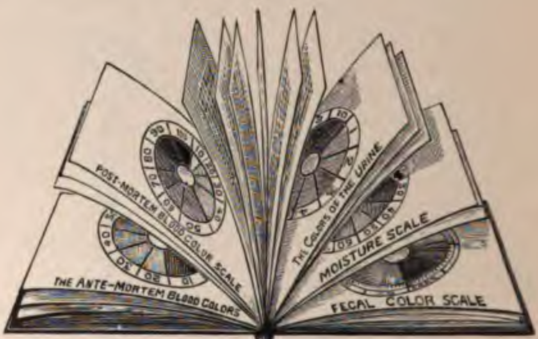

Fig. 231.—Wetherill's hemoglobin scale.

Wetherill's booklet also contains color-scales for postmortem blood, urine, moisture (degree of perspiration), and for the feces.

POLYGLOBULIA.

This is a condition showing, in addition to polycythemia, cyanosis and enlargement of the spleen (usually tubercular). Vaquez was the one first to give an accurate clinical picture of this chronic condition, and at present reports of 30 cases have appeared in the literature.*

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* M. Ascoli, "Riformi Medica," Dec. 21, 1904.

The red blood-cells may reach 9,000,000 to 12,000,000 per c.mm. When leukocytosis exists it concerns the polynuclear cells chiefly. At times mast cells are common and eosinophilia may be present. As a rule, there is an excess of hemoglobin, but in a few instances the hemoglobin was below the normal. The specific gravity is increased and has reached 1.080.

FICKER'S TYPHOID REACTION.

This reaction depends upon the clumping of dead typhoid bacilli. Three small tubes are partially filled with a fixed quantity of bouillon culture.

Method.—Examine each tube to determine that the serum it contains is slightly cloudy, due to bacilli in suspension. To the first tube add a certain quantity of blood-serum (dilution 1–5); to the second add the same quantity of dilute serum (1–10). The third tube is used as a control. The reaction consists in a clearing of the liquid and the formation of a precipitate in tubes one and two, while tube three remains unchanged.

LEISHMAN-DONOVAN BODY.

A questionable parasite first well described by Surgeon Leishman and later detected in persons suffering with "dum-dum" fever, kala-azar, Delhi sore, and tropical anemia by Donovan, Jas. H. Wright and others.

The Parasite.—As generally seen it is of an elliptic shape, varying from 1½ to 4 microns in length by from 1½ to 2 microns in breadth. The smaller bodies may be more fusiform in contour; the larger ones more nearly circular. Christophers compares its shape to a closed cockleshell. A characteristic feature is its double nucleus. All except the smallest forms present a rather large oval nucleus near the periphery and another small rod-

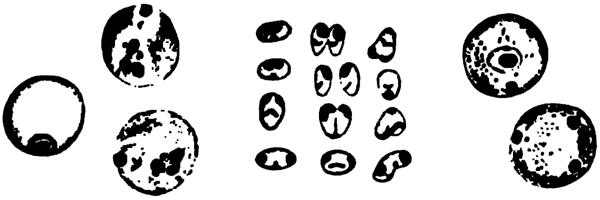

Fig. 232.—Leishman-Donovan bodies.

like nucleus on the opposite side (Fig. 232). In this respect the parasite resembles the degenerated forms of the trypanosome.

The parasite stains by the various modifications of Romanowski's method, and then a well-formed capsular wall is apparent. The larger parasites often display vacuoles and their body substance is stained a bluish-pink color. Christophers has observed the larger nuclei dividing, but the rod-like body remains unchanged. Large spheric bodies containing what appears to be dividing parasites are also seen. Bodies of this type are seen to contain from 3 to 18 small parasites.

Leonard Rogers* has cultivated the Leishman-Donovan parasite on artificial medium at a temperature of 22° C. Other observers have found the parasite to develop luxuriantly at a temperature of 18° C. Rogers states that he has developed a trypanosome from these bodies of Leishman-Donovan; and Chatterjee has confirmed the work of Rogers.† This trypanosome resembles that originally described by Keysselitz (see trypanosome, p. 151). Stimulated by Schaudinn's observation that the intracorpuscular pigmented parasite becomes a trypanosome, we should consider the many possibilities of this Leishman-Donovan parasite playing some part in the rôle of atypical malaria.

Detection of the Parasite.—I have been able to find but two references to the parasite's detection in the peripheral circulating blood. Nearly all authorities agree that the parasites are readily detected in the fresh blood obtained by puncture of the spleen. Splenic puncture is said to be extremely common among general practitioners of the tropics, but in subtropical districts such procedure should be conducted by the surgeon.

Smear the fresh blood thinly upon a slide, add a cover-glass, and study under a $\frac{1}{12}$ oil-immersion objective, as with the malarial parasite (see *Malaria*, p. 157).

URINE.

RAVOLD'S‡ COMBINED HEAT AND CONTACT TEST FOR ALBUMIN.

Reagent.—Dissolve 2 gm. of corrosive sublimate; 4 gm. of succinic acid, and 4 gm. of common salt in 50 c.c. of water. After standing for a few hours add 50 c.c. of a saturated solution of magnesium sulphate; shake to effect a perfect mixture and the reagent is ready for use. Keep in a glass-stoppered bottle.

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* "Brit. Med. Journ.," Sept. 17, 1904, p. 648.

† "Lancet," Jan. 7, 1905.

‡ "Jour. Mo. State Med. Assoc'n," April, 1905.

1. Unless clear the urine should be filtered, and to 5 c.c. of the filtrate add 1 c.c. of acetic acid.
2. Fill a test-tube to one or two inches in depth with the reagent.
3. Add, by means of a pipette, ½ to 1 c.c. of acidified urine (permitting the urine to float upon the reagent). A ring of albumin may appear at the zone of contact of the urine with the reagent.
4. Heat gradually to boiling. The ring thus produced tends to remain.

Ravold has found this test of value for the detection of minute amounts of albumin. Ravold's reagent may be heated in a test-tube and applied after the author's contact method, p. 207.

CLINICAL SIGNIFICANCE OF ALBUMIN.

For convenience of study albuminuria may be considered under the following subheads:

1. Renal albuminuria,—a condition where the albuminous body escapes with the urine into the uriniferous tubules, or, less often, enters the urinary tract at the kidney's pelvis. In either case the condition inducing such escape of albumin is inflammatory. During the early stage of acute nephritis the amount of albumin is very large, and may equal from one-tenth to one-half the quantity of the urine by bulk. A large amount of albumin is also present during the entire course of chronic parenchymatous nephritis; the amount of albumin ranging inversely to the quantity of urine. Toxic albuminuria indicates a condition where albuminuria follows large doses of renal irritants, or where it develops during the course of acute infections (diphtheria, scarlet fever, typhoid fever, etc.).

Throughout the course of chronic interstitial nephritis, and also where there exists amyloid disease of the kidneys, the amount of albumin in a given quantity of urine is relatively small; but when one considers the quantity of urine voided during the twenty-four hours and the percentage amount of albumin correlatively, it will be seen that considerable albumin is lost daily. During an acute exacerbation of chronic nephritis the quantity of urine is also small and the amount of albumin relatively large.

Traumatism in the region of the kidneys, pressure, and massage of the loins and abdomen may cause albuminuria.

2. Albumin may escape into the urine as a result of blood dyscrasia, *e. g.*, leukemia and the severer secondary anemias, and particularly after chronic mineral poisoning.

3. Parasitic albuminuria is seen during infection with the filaria, schistosoma, hematobium, and other parasites (see pp. 292 to 300).

4. Inflammatory processes of the bladder, prostate, and urethra are frequent sources of albumin. It may at times depend upon the escape of seminal fluid with the urine and is usually present in the first urine voided by the male after intercourse. In the female it is very common to find albuminous bodies present in the urine voided by the patient; while the urine obtained by catheter is albumin-free.

6. Intermittent, cyclic, transitory and digestive albuminuria concerns us only in that persons suffering from any one of these forms of albuminuria will in the majority of instances develop true albuminuria later. Here the albumin is of renal origin and suggests an unbalancing of the functions of metabolism and renal secretion.

TORFUGATION.

Wetherill's centrifugal method depends upon the revolution of a tube-containing-bar upon a twisted cord (Fig. 233). The motions are to and fro, and a speed of 5000 revolutions a minute may be obtained. This method provides a most practical means for the recovery of urinary sediments; and for the rapid estimation of albumin by the Esbach method; and for centrifugal anal-

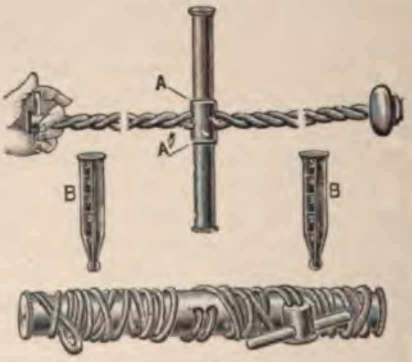

Fig. 233.—Torfuge.

ysis in general (except milk). The torfuge has found a useful field in clinical laboratory methods (see torkrit, p. 523).

QUANTITATIVE ESTIMATION OF PURIN IN THE URINE.

The purin bodies excreted with the urine include combinations containing the nucleus C_5N_4 (see *Uric Acid*, page 196). For convenience of study the purin bodies of the urine are classified into ***exogenous*** and ***endogenous*** purins, the former being derived from the foods, and therefore bearing a direct relation to the amount of purins taken as food. The endogenous purins are thought to result from the nucleins of the body, and therefore their estimation serves as an indication of the degree of disintegration resulting from metabolism.

Method of Determination.—Camerer offers the following method for the determination of the amount of purin bodies in the urine:

Reagents:

1.	Ludwig's magnesia mixture*	100 c.c.
	Ammonia (20 per cent.)	100 c.c.
	Talcum	10 gm.
2.	Silver nitrate	1 gm.
	Ammonia (pure)	100 c.c.
	Talcum	5 gm.
	Distilled water	100 c.c.

Instrument.—The purinometer (Fig. 230) consists of a graduated cylinder which is divided equally into halves, these halves being separated by a perforated cork.

1. Place 90 c.c. of the mixed twenty-four hours' urine, free from albumin, and 20 c.c. of reagent into the superior half of the purinometer.

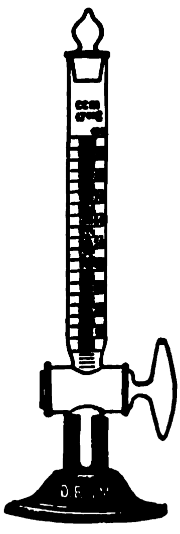

Fig. 234.—Purinometer.

The object of this procedure is to throw out of solution the urinary phosphates which collect at the bottom of the tube, upon the upper surface of the cork.

2. After ten minutes turn the cork and permit the sedimented phosphates to pass into the lower half of the purinometer, and then return the cork to its former position.

3. Add to the remaining urine in the superior half of the purinometer reagent 2, filling to the graduation 100. A precipitate results which is composed of silver chlorid and silver purin; but

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* Crystalline magnesium chlorid, 100 gm.; Water, 1000 c.c.; Ammonia, sufficient to give the solution a strong odor; and Ammonium chlorid, enough to dissolve all precipitate that may result.

the former, silver chlorid, is dissolved by the excess of ammonia present.

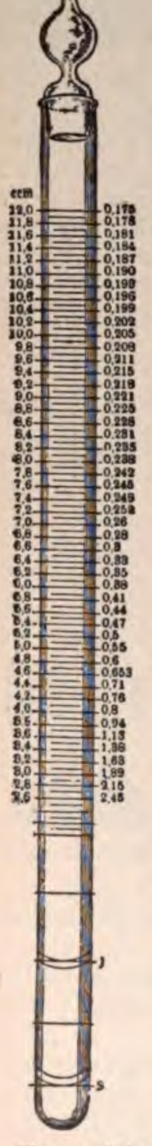

Fig. 235.—Ruhemann's uricometer.

4. The precipitation of the purin bodies present in the urine is hastened by agitating the liquid after the addition of reagent 2. Whenever the yellow silver urine precipitate is seen to contain white flocculi, add a few drops of ammonia to the mixture.

5. Place the purinometer in a dark room for from one to twenty-four hours, after which it should be brought to the light and the precipitated silver purin read from the graduation upon the instrument.

6. Multiply the number of centimeters of the precipitate by 1.5 (percentage), and the empyric value, approximately 0.0010 (which is determined for each apparatus), gives the percentage of nitrogen. The figure thus obtained, when multiplied by the daily amount of urine expressed in cubic centimeters, and this product divided by 100, will give the total amount of purin nitrogen excreted during the twenty-four hours. With each purinometer is furnished a special table by the aid of which the percentage of purin nitrogen is readily estimated. It has been found that the most satisfactory results are to be obtained with urines of a specific gravity between 1.015 and 1.025; and it has been further found that the ingestion of both tea and coffee interferes with the estimation of purin nitrogen; and that a diet composed of eggs, milk, rice, potatoes, bread, butter, and cheese is best adapted for the determination of the endogenous purin excreted. The exogenous purin may be determined while the patient is taking indefinite quantities of meat.

QUANTITATIVE ESTIMATION OF URIC ACID.

Ruhemann's method for estimating the urinary uric acid is dependent upon the union of uric acid with iodin. The instrument employed is termed a uricometer (Fig. 231), and consists of a graduated glass tube 25 centimeters in length.

Reagents.—1. Sulphid of carbon. 2. Iodin solution.

Potassium iodid	1.5	parts
Iodin	1.5	"
Alcohol (absolute)	15	"
Water	185	"

1. Fill the uricometer to mark S with reagent 1.

2. Add 2 c.c. of reagent 2, which fills the uricometer to mark I.

3. Add slowly to the mixture acid urine free from albumin and from sugar, shaking well after each addition. The urine is added cautiously until the brown color gives way to that of white.

4. Read the percentage of uric acid *pro mille* from the right-hand *column* of figures graduated upon the uricometer; and from the left-hand *column* read the number of cubic centimeters of urine employed.

Caution.—Where the amount of uric acid present is too small to register, it is advisable to employ one-half or one-fourth the quantity of iodin solution.

GLYCERIN.

Cammidge's Reaction.—The so-called "Cammidge's reaction" depends upon the recognition of glycerin in the urine, and the test is practically that for glycerose. The local fat necroses probably result from a selective action of the pancreatic secretion. The tissue fat is converted into the fatty acids which readily combine with lime; while the other constituent of the fat molecule, *glycerin*, is thus set free and enters the circulation, appearing in the urine as glycerin, but is converted into glycerose by boiling with a mineral acid. Two tests, "A" and "B," are described in detail for glycerose,* and both are modifications of the phenylhydrazin test for glucose p. 229.

Reaction "A."—1. Place 10 c.c. of filtered (sugar-free) urine into a flask.

2. Add 1 c.c. of strong hydrochloric acid.

3. Place a funnel in the neck of the flask to act as a condenser, and boil gently on a sand-bath for ten minutes.

4. Add 5 c.c. of filtered urine, and 5 c.c. of distilled water; set the mixture in running water and allow to cool.

5. Neutralize the excess of acid with 4 gm. of lead carbonate; add slowly, and allow to stand for five minutes.

6. Filter through a moist filter-paper, and wash the flask upon the filter with 5 c.c. of distilled water.

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* "Lancet," Mar. 19, 1904.

7. To this filtrate add 2 gm. of powdered sodium acetate and 0.75 gm. of phenylhydrazin hydrochlorate; boil for four minutes on a sand-bath.

8. Cool, pour into test-tubes, or, better still, into the tubes of the centrifuge and centrifugalize.

9. Transfer this sediment, by means of a pipet, to a slide, when study under a $\frac{1}{8}$ objective. In the presence of disease of the pancreas this sediment is found composed for the most part of fine needle-like crystals which may appear in dense aggregations or in sheaves.

Fallacies.—This reaction is occasionally obtained with the urine of carcinoma, pneumonia, etc., where there is active tissue change taking place.

Reaction "B."—This differentiating test depends upon the fact that the crystals—end reaction—seen in reaction "A" do not occur in pancreatic inflammations when the urine is first treated with perchlorid of mercury, while such preliminary treatment exerts no influence upon the reaction in cancer of the pancreas, pneumonia, etc.

1. Thoroughly mix 20 c.c. of filtered, sugar-free urine with 10 c.c. of a saturated solution of perchlorid of mercury, and allow to stand for ten minutes.

2. Filter and to 10 c.c. of this filtrate add 1 c.c. of strong hydrochloric acid.

3. Boil for ten minutes in a sand-bath; dilute with 5 c.c. of the filtrate from step 1 and 10 c.c. of distilled water; cool in running water and continue as in reaction "A," step 5.

Clinical Significance.—(*a*) Should no crystals be found by either the "A" or the "B" method, the pancreas is healthy.

(*b*) Should crystals be obtained by the "A" and not by the "B" method, active inflammation of the pancreas exists—an indication for surgical intervention.

(*c*) The solubility of these crystals is studied by permitting acids to flow underneath the cover-glass while observing the crystals under the microscope. Crystals "A" dissolve in 33 per cent. sulphuric acid in one-half to one minute, where acute pancreatic inflammation exists. In chronic inflammation they require two minutes to disappear. Crystals obtained by both "A" and "B" methods are suggestive of pancreatic cancer. In cancer the crystals require five minutes to dissolve.

GASTRIC CONTENTS.

CIPOLLINO'S TEST.*

Reagents: (1) Anilin water; (2) a titrated solution of sodium hypochlorite; (3) a solution of hydrochloric acid 5 parts to 1000, for control tests.

1. Place 2 c.c. of filtered gastric contents into a test-tube.
2. Add one-half c.c. of anilin water and shake the mixture gently.
3. Add drop by drop 4 or 5 drops of the sodium hypochlorite solution, shaking gently after each addition; and should the hydrochloric acid present not equal the normal, a yellow color results. Should the mixture become violet, repeat the test, using 2 parts of gastric fluid with 1 part of distilled water; and a violet color now shows that there is more than 2 parts per 1000 of HCl present—the lower normal quantity. A faint violet color resulting after the employment of gastric fluid and distilled water in equal parts denotes that there is three parts of HCl per 1000—the extreme limit for normal gastric fluid. A dark violet color is indicative of an excess of HCl; and should a faint violet color persist, the HCl is above normal.

PURIN BODIES IN THE FECES.†

According to Schittenhelm, the amount of purin nitrogen in the feces is in direct proportion with the amount of solid substance. This amount is directly influenced by diet; *e. g.*, a diet with much residue or one rich in nucleins (thymus gland) causes an increase. In diarrhea some of the food purins pass into the feces; and in disease of the pancreas due to incomplete digestion of nuclein the amount of purin in the feces is greatly increased.

The purin bodies are decreased in the feces of alcoholism and in constipation. The intestinal wall contains purin bases; viz., adenin, guanin, xanthin, and hypoxanthin; and it has been suggested that a portion of the purins in feces is derived from this source. Meconium contains uric acid but not purins (see ***Purins in Urine***, page 529).

MOISTURE OF THE BREATH.

This is accurately determined by the aid of Wetherill's hygroscope; or by the color scale, page 524.

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* "Riforma Medica," Dec. 7, 1904.

† "Deut. Arch. f. klin. Med.," Bd. lxxxi, p. 423.

Hold the open surface of the instrument or the slip of litmus paper directly in front of the opened lips and direct the patient to exhale through his mouth.

Clinical Significance.—The moisture of the normal breath approximates 85. The degree of moisture is increased in conditions where the skin and kidneys are inactive, and Wetherill found it to reach 95 in uremia.

CLINICAL SIGNIFICANCE OF BUCCAL SECRETION IN DISEASE.

The reaction of the saliva is acid in diabetes, neoplasm of the stomach, in leukemia, pernicious anemia, and occasionally in chlorosis. In uremia it may contain urea. Fleckseder* detected sugar in the saliva of 2 of 13 cases of diabetes studied.

Oligosialia (diminished saliva) is to be seen in diabetes mellitus and insipidus, severe diarrhea, profuse sweating, vomiting, dropsy, cachexia, gastric carcinoma, malignancy, the anemias, hepatic cirrhosis, in high fevers, and rarely after ptyalism. At such times the saliva is cloudy, tenacious, and emits a sweetish odor.

Ptyalism is characterized by a clear, thin, alkaline secretion, which is decreased in rhodan. Ptyalism is met with in stomatitis, pregnancy, painful gastric maladies, gastric ulcer, and nausea. An intermittent increased flow of saliva is observed in neurasthenia, trigeminal neuralgia, and locomotor ataxia.

CYTODIAGNOSIS.

Cytodiagnosis consists in a differential study of the cellular elements found in fluids recovered from the serous sacs (see differential leukocyte count, p. 98).

Clinical Significance.—An excess of lymphocytes (mononucleosis) is probably suggestive of tuberculosis or, more correctly, indicative of the presence of foreign cells in the serous membrane, and that nature is endeavoring to eliminate such offending substance by means of the macrophagocytes, while a preponderance of polynuclear cells points toward suppurative processes or toward a widespread tuberculous or gummatous condition with probable necrotic change. Should the fluid contain many endothelial cells (endotheliosis), tuberculosis is to be favored. Polynucleosis is suggestive of a virulent infection (empyema), and is also to be found in such inflammatory maladies as rheumatism, malignancy, in heatstroke, and infarct of the lung. In transudates, endothelial cells are found in sheets and clusters, and, according to Lewkowiez,* endotheliosis is suggestive of mechanical obstruction, as seen in transudates of heart and kidney disease. Vargas-Suarez found the eosinophils increased in cancer of the pleura and in two cases where the diagnosis was obscure. There may be an excess of eosinophils in the blood, the cause of which is possibly dyspnea. In cancerous involvement of the serous surfaces one would expect to find many endothelial cells in the effusion from involved sacs. Erythrocytosis indicates a hemorrhagic process, and may depend upon a break in the continuity of the blood-vessels or upon an inflammatory process. In two instances in which I recovered bloody fluid from the right pleura, the antemortem diagnosis and later the postmortem diagnosis was cirrhosis of the liver, the pleuræ were normal.

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* "Zentralblatt für Innere Med.," Jan. 14, 1905.

Transuded fluid will be found to contain a few leukocytes and endothelial cells, many of which show evidence of fatty degeneration. In ascitic fluid during the course of myelogenous leukemia many eosinophils and mast cells have been noted. In cases in which the fluid is concealed for a long time crystals of cholesterin and Charcot-Leyden crystals may be found, the former being oftenest found in hydrocele fluids.

Cytodiagnosis of Mumps.—Sicard and Dopter‡ collected saliva directly from the parotid gland by introducing a short piece of a fine sound through the excretory duct of the gland. The saliva from 32 persons free from mumps contained very few cells, but that from 52 persons with mumps was pathologic almost from the onset in 49 instances. By examining the saliva from the gland in this way it is easy to differentiate parotitis at any stage and to reveal the parotitic origin of certain affections of the testicles.

Method.—1. Collect the fluid in a clean bottle and cork tightly until ready to begin its cytologic study.

2. Place the fluid in the centrifuge or the torfuge and sediment for from three to ten minutes.

3. Lift the sediment into a pipet and transfer it to a slide, where spread thinly over a comparatively large surface.

4. Dry this preparation in the air and fix by heat or by the fumes of formalin (see *Fixing Blood*, page 74).

5. Strain for one-half minute with a solution composed of one-half of 1 per cent. of eosin in 70 per cent. alcohol; and counter-

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* "Wien. klin. Woch.," Sept. 1904, p. 978.

† "Beitrage zur. klinik der Tuberkulose," 1904, Bd. ii, p. 201.

‡ "Presse Médicale," March 29, 1905.

stain with a 2 per cent. aqueous solution of methylene-blue, for one-half minute. Wash in water and study under a one-twelfth oil-immersion objective (see *Differential Leukocyte Count*, page 98).

Caution.—Upon standing serous fluids often develop a flocculent coagulum or sediments in which many of the cellular elements become entangled, hence cytologic examination of such fluids is valueless.

EXUDATES.

PARASITES OF SMALLPOX, VACCINIA, AND VARICELLA.

It is quite well established that in the lymph of smallpox, varicella, and vaccinia are to be seen small unicellular bodies (protozoa) which possess a variable degree of ameboid movement.

Parasite.—The organism is a spherical body $\frac{1}{2500}$ of an inch in diameter, containing a rather large central nucleus. This nucleus is somewhat obscured by numerous refractile granules, which are regarded as probable spores. The extra-cellular spore-like bodies are motile unless found in glycerinated calf-lymph. The cytoplasm of the cell is finely granular.

Detection.—1. Collect the lymph from the vesicles of the several diseases and study in a hanging-drop preparation (see Widal reaction, p. 118).

2. Keep the specimen warmed at body-temperature and examine under a one-twelfth oil-immersion objective.

Cautions.—In variola collect the lymph on or before the fifth day of the eruption. Human vaccine lymph should be procured upon the eighth or ninth day. The parasite of chickenpox is demonstrable in lymph gathered the first, second, and third days of the eruption, and is very motile, as is also the parasite of human vaccine. Dilute glycerinated calf-lymph to four times its bulk with normal saline solution.

SEROUS EXUDATES.

Protozoön of Scarlet Fever.—C. W. Duval,* in the study of 18 cases of scarlet fever, produced blisters by the use of ammonia and examined their serum for protozoa-like bodies (Mallory's bodies). In five instances positive results were obtained, and in three of these the spherical segmented forms were observed. Positive results were obtained when the eruption was fully developed.

In one case that succumbed to the malady foci of organisms apparently identical with those detected in the serum of the blisters were found in the skin.

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* "Univ. of Penn. Med. Bul.," Nov., 1904.

CHYLOUS EXUDATES.

DISTINCTIVE FEATURES OF CHYLOUS AND PSEUDOCHYLOUS FLUIDS.

True Chylous Effusion.	Pseudochylous Effusion.
1. The fluid consists of a fine emulsion containing but few cellular elements.	1. A less perfect emulsion containing large numbers of epithelial cells and granular débris which is seen to display the various stages of degeneration, and to contain few fat droplets.
2. Accumulates rapidly after tapping.	2. Collects more slowly, varying with the exciting pathologic cause.
3. Contains sugar.	3. Sugar absent, as a rule.
4. The melting-point of the fat present in the fluid will be found to vary with the melting-point of the fat taken as food.	4. Fatty foods have no effect on the melting fats contained in the fluid.
5. The amount of fat present is in direct relation with the amount of fats ingested.	5. Not affected by diet.
6. On standing a distinct creamy layer does not collect on the surface of the liquid.	6. A layer may collect which varies with the number of oil droplets present. A heavy sediment is liable to collect at the bottom of the liquid.
7. Fluid obtained by paracentesis may emit the odor of foods that have been ingested.	7. Does not emit the odor of foods.

Clinical Significance.—Analysis of reports of 126 cases of milky ascites (2 cases observed personally) gave 24 complicating carcinoma; 17 cases resulting from tuberculosis; 11 cases accompanying cardiovascular conditions; 8 cases due to disease of the liver; 7 cases following puerperal sepsis; and 4 cases the result of congenital cysts.

In addition, there were 3 instances where chylous ascites followed infection with the filaria sanguinis hominis. Obstruction to the thoracic duct was observed postmortem in 11 of the cases, and the duct or receptaculum was found to be ruptured as the result of traumatism in 7 of the cases.

Sixty-one of the cases were females, 50 males, and in the remaining reports the sex was not given. A further analysis as to the number of cases occurring at the various periods in life gave 6 under one year of age; 3 between one and five years; 13 between five and ten years; 9 between ten and twenty years; 12 between twenty and thirty years; 34 between thirty and fifty years; and 24 after the age of fifty.

Milky fluid is also met with in the pleural and pericardial sacs.

CYTOLOGIC STUDY OF SPINAL FLUID.

Sicard, Widal, Ranout, and Wieder and Mamlock have contributed elaborate papers upon the clinical values of cytodiagnosis of the spinal fluid in diseases of the meninges, brain, and cord. These studies have even included the most varied affections of the nervous system, and the point of special interest appears to be an increase in the relative proportion of small mononuclear leukocytes (lymphocytes). The lymphocytes have been found increased in tabes, progressive paralysis, cerebrospinal syphilis, syphilitic hemiplegia, herpes zoster, and sciatica. The lymphocytes are not increased in syringomyelia, poliomyelitis, polyneuritis, hemiplegia of old age, compression myelitis, functional neuroses, epilepsy, and cerebral tumor.

Cases of general paralysis, tabes and those of syphilitic origin provide the most constant findings (mononucleosis); but in these the findings are often confusing, giving an increase in the percentage of lymphocytes at one examination and a normal or decreased number at another. In uremia with convulsions a moderate increase in the mononuclear cells is common; while in tetanus the increase is likely to be more decided, and may be associated with many polymorphonuclear leukocytes. Brain tumor and tumor of the cervical cord have shown a decided increase in mononuclear cells.

The number of contradictory statements regarding cytologic study of the spinal fluid makes it difficult for one to arrive at definite deductions. This difference in the results obtained by different observers is dependent in part at least upon the varied technique employed, and also because there was no set schedule observed as being normal for the cerebrospinal fluid of man (see *Differential Leukocyte Count*, page 98).

INDICAN IN THE URINE.

The following test has proved very satisfactory in the hands of Dr. Daland: To 10 c.c. of filtered urine add one drop of a 1 per cent. potassium chlorate solution, then 5 c.c. of chloroform, and, lastly, 10 c.c. of pure fuming hydrochloric acid (specific gravity 1.19). It is very necessary that the reagents should be added in the order given.

Mix thoroughly by pouring repeatedly from one test-tube to another. By this method the indican (indoxyl-potassium-sulphate) is reduced to indigo which dissolves in the chloroform and imparts a blue color to it. In about ten minutes the maximum coloration has been reached and the whole liquid should again be thoroughly mixed. The chloroform will be colored more or less blue according to the amount of indigo set free.

If the urine contains iodids, the chloroform will be colored violet. This violet color may be removed by adding three drops of a 5 per cent. aqueous solution of sodium thiosulphate, whereupon the blue coloration, if present, will appear.

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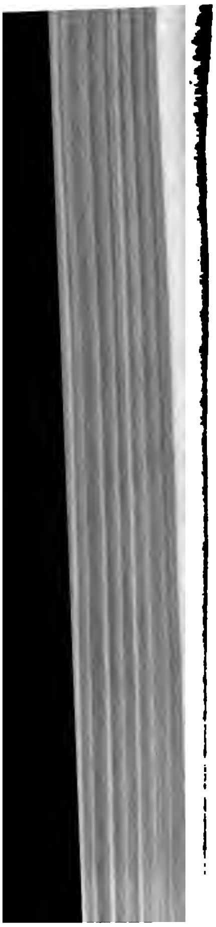

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